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(54) Title: PATTERN FORMATION, REPLICATION, FABRICATION AND DEVICES THEREBY

(57) Abstract

Methods for the formation and replication of patterns are disclosed, with objectives including economical microfabrication and nanofabrication, suitable for both low and high volume production. A patterned relief is formed on a surface by various techniques, which is then replicated on daughter surfaces. Methods for the reduction or elimination of defects are included. Various compositions may be patterned to address a wide range of applications. Spatial control over copolymer synthesis, synthesis of patterned molecular monolayers and multilayers, fabrication of microelectronic, microelectromechanical and microfluidic devices may be effected by the methods of the present invention. Various articles of manufacture and uses thereof, including improved methods and means for genome analysis, are further disclosed.

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PATTERN FORMATION, REPLICATION, FABRICATION AND DEVICES THEREBY

Field of the Invention:

The invention relates to the fabrication of two- and three-dimensional patterns and the production of devices thereby; the present invention finds application in the areas of microelectronics, micromechanics, microfluidics, scanning probe microscopy, mass data storage, scientific instrumentation, clinical diagnostics, molecular assembly, and other areas.

Related Art:

Microfabrication and Microelectronics:

The field of integrated microelectronics depends critically upon the generation of predetermined two dimensional patterns. Generally, some lithographic process (e.g. involving actinic radiation) is employed to transfer a pattern from a mask to a resist, which is developed to expose portions of the surface of the underlying substrate. Such exposure permits the patterned etching of the exposed surface, or the patterned diffusion of impurities into regions near said surface. Thus, metal layers may be etched to form wiring patterns and regions of a semiconducting substrate may be doped to form electronic components in an integrated device. Metal layers may also be patterned by lift-off processes involving the local dissolution of a pre-patterned resist underlayer[1], which carries away with it the immediately overlying regions of the metal layer. Such resist masking and modification steps are repeated with different mask patterns and different modifications are performed which spatially

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vary according to the pattern formed in said resist. The alignment of
mask patterns during each step with respect to all other steps is a
critical limit on fabrication resolution. A summary of the basic
methods is given is described by Millman[2]. Microfabrication of
5   integrated optics devices relies on similar techniques, as well as
further additional techniques, a discussion of which is given by H.
Nishihara, M. Haruna and T. Suhara.[3]

Fault Tolerant Circuit Designs

10   One approach that has been applied in the field of microelectronics
to increasing the tolerable physical defect rate arising from a
particular fabrication process involves fault tolerant circuit design.
This is a category of methods which includes redundancy of functions
or components, self-testing or quality checking during the fabrication
15   process, and rerouting of connections between functional blocks to
serve functions lost to defects. For example, such methods have been
applied to dynamic RAM fabrication, where the memory bit array is
divided into a number of blocks, the total capacity of which is larger
than the device specification. All blocks are checked for defects or
20   functionality, and where defects are found, the involved blocks are
deactivated. Functional blocks are then reconfigured, as needed, to
contiguously fill the device address space. Analogous methods have
been applied in the design of gate arrays, field programmable gate
arrays, memory devices and other microelectronic devices.
25

Microscale and Nanoscale Lithographic Methods:
Defect Reduction by Resist "Voting":

In U.S. Patent Number 5,308,722, J.L. Nistler has disclosed a
method for the reduction of defects in lithographic phase shift masks.
30   The essence of this method is to form a resist pattern, use it to etch
the underlying quartz surface only partially, remove said resist
pattern and any defects it may include, form a substantially identical
pattern of resist on said quartz surface again, partially etch again
and remove said substantially identical pattern of resist again, in
35   repetitive cycles. Thus, only etch resistant regions appearing in all
resist patterns will be reproduced in the final patterned quartz
article at the fully etched depth. Conceptually, this method checks
one pattern, which may contain random defects, against other patterns

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which may also contain random defects which are not likely to appear
at the same location as those random defects in any other pattern. In
the case of the phase shifting reticles produced by that invention,
only regions etched such that the transmitted light is shifted by 180

5   degrees exposes the lithographic resist these reticles are used to
expose. Thus, correspondence between multiple source patterns is
required for a feature to be represented in the final product mask
produced therefrom.

10   Contact Printing of Resist by Lithographic Plate:
    In U.S. Patent Number 5,380,620, T. Suzuki and F. Shinozaki teach a
method whereby a lithographic plate comprising regions of ink binding
and ink repelling regions is contacted with an ink or resist sheet and
then contacted with a substrate to which said ink or resist which

15 bound to said ink binding regions of said ink. This method forms
lithographic plates by exposing materials similar or identical to
those used as resists in conventional microfabrication, such that
exposed regions have affinity or repel ink or other liquids, such that
a pattern of differential retention of ink or said other liquids may

20 be used to form a pattern of said ink or other liquids on said
lithographic plates and then transfer this pattern to the substrate.
While this method reduces the extent to which photolithographic
equipment is needed for patterning, production of micropatterned
articles by this method still requires routine access to such

25 photolithographic equipment for lithographic plate production.
Further, primary reliance on differences in affinity of liquids to
those regions of said lithographic plate treated to have such affinity
compared to the affinity of said liquids to the substrate to be thus
imprinted entails that this method will require considerable effort

30 for optimization when applied to new inks or resists to different
surfaces.

            Photolithography Generated Relief Patterns,
            Mechanical Transfer Thereof, and Use as Lithographic
35   Plate:
    Methods for the production and transfer of relief patterns in
polymeric resist materials or variants thereof are reviewed by B.
Bednar, J. Kralicek and J. Zachoval.[4] A relief is produced by

selectively exposing regions of a photoresist coated onto a first
surface and mechanically transferring either the exposed or non-
exposed regions to a polymeric foil second surface by juxtaposing said
second surface to the image-exposed polymeric resist and relying on
5   the differential adhesion properties resulting from exposure. Thus, a
positive image is produced on one surface while the other surface
retains the corresponding negative pattern. Such relief patterns may
provide differential wetting or liquid-retention properties differing
from those of the underlying surfaces, and thus be used as offset
10   lithographic plates.

Differential Resist and Differential Ion Milling Thereby:

G. Gal has, in U.S. Patent Number 5,310,623, taught a method
15   whereby microlens arrays are formed. In this method, greyscale
exposure of a resist material may be used to cause the differential
etching (in the preferred case by ion milling) of the underlying
substrate, to produce a correspondingly curved surface. (Note that
this inventor uses the term replica in a different sense than that
20   used herein.) Greyscale exposure results in a different effective
etch protection by the resist, according to the degree of exposure.
This may be termed etch-resistance density or depth dependent etching.

Microelectromechanical Systems:

25   Microfabrication techniques have been applied to multilayered
substrates comprising sacrificial layers which permit under-etching.
Under-etching permits the fabrication of structures with suspended or
overhanging members and freely moving microscale mechanical parts.
Such a process may be combined with conventional microfabrication
30   steps or applied to substrates comprising microelectronic devices to
integrate both electronic and mechanical components of micron and
submicron dimension. Such integrated devices have been termed
microelectromechanical systems (MEMS)[5].

Microfabricated Scanning Probe Microscopes and Actuators:

J.J. Yao, N.C. MacDonald and S.C. Arney have described
microfabricated actuators, springs and STM instruments[6], and have

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taught methods for producing components of these in U.S. Patent
Numbers 5,179,499 and 5,235,187. These capacitor actuators have
nanometer scale accuracy and high resonant frequencies.

S. Akamine, C. Quate and co-workers[7] have described a different
5 microfabricated STM design relying on bimorph piezoelectric actuators.

Data Storage with Scanning Probe Devices:

The high detection resolution achieved by the various regimes of
scanning probe microscopy as well as the ability to modify surfaces
10 and structures thereupon with these instruments has motivated much
interest in the use of scanning probe technology for data storage and
retrieval. It is anticipated that means such as these will be
necessary to surpass the physical limits on recording density in
magnetic and far-field optical surface recording technologies.

15 Recently, T.C. Reiley, L.-S. Fan and H.J. Mamin[8] have demonstrated
the recording and readout of data using and AFM based instrument and
the polycarbonate coated surface of a transparent disk as a storage
medium. Data is recorded in the form of pits on said surface. A
laser is used to heat said polycarbonate above the glass transition
20 temperature, and the AFM tip impresses a pit into the locally heated
region. The size of the resulting pit corresponds to the apical
geometry of said AFM tip. Readout is accomplished by conventional
contact-mode AFM detection of said pits. Thus, upon recording of a
bit pattern onto such a polymer coated surface, a relief pattern is
25 formed which encodes the corresponding, stored data. These workers
have formed pits as small as 100nm across and 10nm deep, yielding an
areal storage density of 25 Gb/in^2.

R. Imura, S. Hosaka, et al.[9] have also recently demonstrated a
scanning probe technology based data storage method, also using an
30 AFM-like instrument with a gold-coated AFM tip. These workers rely on
filed evaporation of metal from said gold-coated AFM tip to form dots
(representing data bits) on a silicon surface, with readout again
accomplished by conventional contact-mode AFM.

In a somewhat different approach, R.E. Betzig et al., in U.S.
35 Patent Number 5,286,971 and elsewhere[10], teach a method by which data
may be stored by employing a medium which undergoes physical
transformation upon exposure to light and exposing said medium to
light according to a near-field transfer process, such that the region

of said medium exposed is limited by the physical dimensions of the
aperture transmitting said light rather than the wavelength of said
light, where said physical transformation includes a local change in
optical properties and where said local change in optical properties
5   is detectable by a method similar to Near Field Scanning Optical
Microscopy (NFSOM). In this method, density advantage is gained
compared to the wavelength related density limitations of far-field
optical recording methods, with densities as high as 45 Gb/in^2 having
been demonstrated .
10

Scanning Probe Pattern Formation:
Scanning Resist Patterning:

Scanning Tunneling Microscopes (STM)[11,12], Atomic Force Microscopes
(AFM) and Near Field Scanning Optical Microscopes (NFSOM)[13] have been
15   used to selectively form patterns in surfaces on the nanometer scale.
These include patterns in materials or layers which mask the
underlying substrate from the action of etchants when not modified by
the microscopic probe used.

20   ### Chemical Manipulation and Synthesis with Scanning
Probe Microscopes:

W.T. Müller, P.G. Schultz et al.[14] describe a patterning process
whereby a surface is adsorbed with molecules comprising a chemical
functional group which may be transformed to a second chemical
25   functionality by contact with a metal catalyst, an AFM is used to scan
a tip coated with said metal catalyst over areas of said surface which
are intended to be transformed, and said chemical functional groups
transformed by the resulting, spatially limited catalytic process are
then subjected to further chemical reactions which are selective for
30   the catalytic reaction product second chemical functional groups.
B.J. McIntyre, M. Salmeron and G.A. Somorjai[15] have similarly effected
positional control of metallic surface catalysis using a platinum-
rhodium STM tip in an atmospheric-pressure chemical reactor to
catalyze the rehydrogenation of molecules comprising alkyne
35   functionalities on (111) platinum surfaces.

M.A. Voelker[16] has proposed a method for the patterning of chemical
moieties on surfaces, and the formation of patterns in multicomponent
Langmuir-Blodgett (L-B) films according to the interaction of affinity

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groups of molecules in said L-B films with complementary affinity
moieties on said surfaces. Such a method would entail that the
molecules of which said L-B film is composed have good mobility in the
as-formed L-B film prior to any polymerization, and that transport

5   permits good segregation of molecular species within said L-B film
without any congestion problems or other hindrance to the proper
assortment of species to match the pattern on the underlying surface.
Because said L-B film is only exposed to said pattern after deposition
onto said surface, the mobility of molecular species within said film

10   will be reduced as compared to mobility in the undeposited film, and
this will reduce the efficacy of the proposed method. Further, though
the proposed method provides for multiple uses of the initial
patterned surface, it does not provide for the replication of the L-B
layer patterns thus formed except where these comprise multilayers

15   which remain associated together. In other words, this method only
provides for the additive accumulation of patterned L-B layers,
whether these are then isolated from an initial surface or formed
successively in a multilayer molecular based structure.

20   **Microfabrication with Replication:**
     Integrated optical devices and other microscale optical components
have been fabricated by the replication of relief patterns into
polymeric materials[17]. The optical properties of these materials and
the structures resulting from pattern replication determine device

25   function and characteristics. Replication methods used include
injection molding and casting into polymeric, elastiomeric or metallic
molds or mold inserts, and hot embossing with reliefs, for example,
metal reliefs. Multiple cycles of replication may be performed to
yield a large number of reliefs for the rapid mass production of

30   devices, according to the fidelity of replication and acceptable
device tolerances.

     Over the last decade, methods have been developed to form three
dimensional patterns on the micron and sub-micron scales, combining

35   lithography with electroforming, micromolding and mold replication.
An original microrelief is formed by the lithographic depth patterning
of a relief material, which is then developed to form the desired
predetermined structure (first relief). Electroforming is then

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performed to yield a metal negative mold insert conforming to the
polymer resist structure, and then separated from the original resist.
This first generation mold may then be as a mold for the casting or
microinjection molding of a second polymeric relief nearly identical
5   to the first relief. The molding or casting process may be repeated
with the first generation mold, and the electroforming process may be
repeated with second or subsequent generation polymeric relief
structures produced with said molds by appropriate methods. Thus,
polymeric objects and metallic objects with sub-micron patterns may be
10   economically reproduced. This set of methods, known as LIGA, was
developed by E.W. Becker et al.,.[18] These workers reported
reproduction of lateral features smaller than 0.1 micron. These
methods are particularly useful in the production of devices with
microscale features and moving parts. Where the lithographic method
15   employed is synchrotron X-Ray lithography, high aspect ratios may be
achieved with resists of up to millimeter depth. More recently, M.
Abraham et al.[19] have reported the fabrication of micro-optical
systems with LIGA, and further extend these methods by using mold
inserts thus produced for hot embossing.

20   Similarly, M.T. Gale et al.,[20] extend methods from the embossed
diffractive foil and compact disk (CD) industries by the replication
of an original (e.g. microfabricated) microrelief on the surface of a
replication shim through electroforming, followed by replication of
this first shim by surface passivation followed again by
25   electroforming. These workers find that the shim replication process
occurs with only slight (<2nm) increases in surface roughness per
generation, thus enabling the reproduction of nanoscale relief
features, with corresponding cost advantages. Related injection
molding methods are described by A. Neyer et al.,[21] and R. Klein and
30   A. Neyer.[22]

         **Micromolding of Ceramics and Resonators
         Therefrom:**

         J.A. Bride et al.[23] have shown that micropatterned reliefs may be
35   used to micromold ceramic patterns. Specifically, these workers used
a polyimide relief, prepared by reactive ion etching through a Ti
mask, was used as a "cookie cutter" which was impressed into a
chemically softened ethyl-methacrylate tape comprising powdered

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ceramic particles (ceria-zirconia) of average size 0.3 microns. After embossing, the tape was dried, removed from the micromold, heated at 600 degrees centigrade to remove the EMA binder and then sintered at 1500 degrees centigrade. Features as small as 4 microns could be thus
5   produced. These workers have developed this method for the fabrication of piezoelectric ceramics.

Self-Assembling Monolayers and Resists Therefrom:

10 A paradigmatic example of self-assembling monolayers is provided by the self assembly into monolayers of alkanethiols onto gold surfaces, according to the strong affinity of thiol groups for gold surfaces.

As reviewed by A. Ulman,[24] these self-assembling monolayer systems also include organosilanes and derivatives thereof (e.g. $RSiX_3$, R_2SiX_2
15 or R_3SiX where R groups may be identical or distinct), which form covalent linkages to surface hydroxyl groups (e.g. formed on surfaces with exposed SiO_2 including native silicon oxides, SnO_2, TiO_2). Note that according to appropriate chemistries, monolayers thus formed may be modified such that the molecules of said monolayers thus formed
20 bear terminal hydroxyl groups, which may then serve as donors for the successive, covalent addition of monolayers, forming crosslinked multilayered structures on said surfaces. Note also that the organic chains of such monolayers may additionally comprise polymerizable or photopolymerizable groups such as alkynes.[25] Further, such monolayers
25 may be used to render surfaces hydrophobic, such that they are protected from a solution with which said surfaces are contacted, such as an aqueous etchant solution.

Simple Methods for Initial Patterning of Monolayer
30   Resists:

Several groups have formed patterns in homogenous self-assembling monolayers (SAMs) formed on metal surfaces or surfaces of metal films, generally gold films, by methods related to milling or grinding. These methods include scraping with sharpened tools[26] to produce the
35 desired pattern as well as applying increased force to an AFM tip during scanning operations or increasing the setpoint current to cause physical contact of an STM tip so said metal surface with increased local forces resulting.

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G.M. Whitesides and co-workers[27] have also produced a rapidograph-like micropen for the application of organothiol compounds to specified surface regions, and used this writing instrument to produce organothiol patterns on gold surfaces.

5

Microcontact Printing of Self-Assembling Monolayer Resists:

G.M. Whitesides and co-workers have disclosed methods for the microcontact printing of compounds which form SAMs onto surfaces

10   coated with evaporated metal films, with a strong emphasis on organothiol compounds used in conjunction with evaporated gold film coated surfaces. These workers have demonstrated that metal regions coated by said monolayers are resistant to wet chemical etch solutions. After such a chemical etch step, the underlying surface is

15   exposed; where said underlying surface is, for example, crystalline silicon, it may be wet etched to produce a relief pattern corresponding to the mirror image of the relief surface used in said printing. Microcontact printing methods used by these workers has been based upon relief patterns replicated in elastiomeric polymer

20   surfaces, generally using (PDMS) elastiomers and reliefs replicated from microfabricated surfaces. These methods are found by these workers to yield a defect rate (of over $1/mm^2$ per mask-etch cycle)[28] which they deem unacceptably high to make these methods competitive with conventional lithographic techniques for the production of

25   microelectronic devices. They speculate that these defects primarily owe to microcrystallites in said gold films, arising from incomplete coverage of said microcrystallites by said organothiol molecules.

Patterns of organothiol SAMs by these methods have reproducible resolution of features smaller than 100nm. Such patterns have also

30   been formed on the surfaces of glass cylinders and fibers, which have been used subsequently to replicate the corresponding pattern on flat surfaces by a rolling technique, analogous to that used in the printing of holograms in metallized polymeric films but on a smaller scale.

35

Wettability control by Self-Assembling Monolayer Composition and Structures Thereby:

G.M. Whitesides and co-workers have further demonstrated that
patterns of such organothiols affect the wettability of the surface
regions they coat, and that the presence of a pattern of a first
organothiol can mask the adsorption of a second organothiol, such that
5   when a pattern of a first organothiol is disposed upon a gold surface
and said gold surface is then contacted with a solution of a second
organothiol, said second organothiol forms a SAM only on exposed areas
of said gold surface; the resulting surface is thus completely coated
with two complementarily patterned organothiol SAMs. Thus, said first
10   organothiol may have a terminal methyl group such that regions with a
SAM of said first organothiol composition are wetted well by organic
liquids and poorly by aqueous or polar solutions, and said second
organothiol may have a polar terminal group, such as a hydroxyl group
such that regions with a SAM of said second organothiol are wetted
15   well by aqueous or polar liquids and poorly by organic liquids.

With appropriate patterns thus formed, the patterned wettability of
such surfaces has been used to fabricate surface-tension defined
structures, such as a microlens array[29] from solutions comprising
optically clear polymerizable compounds. Wetting may be conducted,
20   for example, by vapor condensation or dipping into solutions.

Lithographically Patterned Copolymer Synthesis:

S.P.A. Fodor et al.[30] have disclosed methods for the spatially
directed synthesis of copolymers including biopolymers such as
25   oligonucleotides and polypeptides. These workers bind initiator
chemical groups protected with photolabile protecting groups to a
substrate. Spatially masked exposure to appropriate wavelength
actinic radiation removes said photolabile protecting groups from
exposed regions, where the extent of said exposed regions is delimited
30   by an exposure mask having a desired radiation masking pattern. Said
surface is then contacted with a solution of photolabile protecting
group protected monomers of desired composition, which react only with
the deprotected (photodeprotected) initiator chemical groups located
at exposed surface regions. Unbound monomers are then washed. Said
35   masked exposure step, said protected monomer contacting steps and said
washing steps are repeated so as to synthesize desired oligomers at
desired locations on said surface such that an array of diverse
oligomers situated on said surface is produced and the location of an

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array element comprising oligomers bears a predetermined relationship
to the sequence composition of said oligomer copolymer located at said
array element. Such copolymer arrays may be used, depending on the
compositions of said copolymer, to detect affinity binding to members

5   of a population of molecules (comprised within the composition of said
array) of a sample, which methods are useful to screen antibody
specificity, to detect the presence of polynucleotide sequences in a
sample or mixture, and for sequencing by hybridization methods.

Other methods for the production of similar copolymer arrays have

10   been demonstrated by others, albeit generally at lesser array element
densities.

Array Fabrication by Masking for Materials Screening:

15   X.-D. Xiang et al.[31] have recently described a method for the
screening of materials comprising the repetitive evaporation of
different materials onto a surface occluded by a mask, such that an
array is formed comprising elements of different composition, which
array is then sintered. Array elements thus prepared may then be

20   subjected to screening for desired properties in analogy to the
methods of the field of combinatorial chemistry.

Microcontact Patterning of Proteinaceous Arrays on Surfaces:

25   Whitesides and co-workers[32] have used elastiomeric patterned relief
surfaces to produce surfaces with correspondingly patterned deposits
of proteins. In this method, a gold surface is patterned with an
organothiol, and then exposed to and organothiol comprising terminal
hydrophilic moieties are then adsorbed to unpatterned regions. Such a

30   surface is then contacted with a solution of the desired protein or
proteins, which adsorb to the hydrophobic surface regions. Such a
method does not provide for the formation of patterns of variations of
combinations of proteins from one region to the next.

35   ### Production of Molecular Multilayers with Monolayer Precision:

Various workers[33,34,35] have described methods for the stepwise
addition of monolayers or bilayers to a surface, such that a

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multilayer structure comprising a predetermined number of layers of only a few nanometers in thickness may be formed. According to these methods, once a monolayer or bilayer is thus deposited on a surface (including surfaces comprising monolayers, bilayers or multilayers), 5 said monolayer or bilayer remains situated upon said surface.

Synthesis of Sheet-like Polymers:

S.I. Stupp et al.[36] have shown that substantially linear molecules comprising extended linear regions along their length, a polar 10 enantiomeric ordering center and two polymerizable reactive groups of distinct polymerization chemistry at a specific positions along their length spontaneously assemble into layered mesoscale structures which may then be polymerized to yield two dimensional polymers. This work continues the teachings of S.I. Stupp disclosed in U.S. Patent Number 15 5,229,474.

Terminally Functionalized Rod-like Molecules:

M. Kotera, J.-M. Lenh, and J.-P. Vigneron[37] have disclosed compounds comprising rigid rod molecular segments with single terminal 20 nucleobase moieties. Said compounds self assemble in organic solvents to form supramolecular rods according to well-known Watson-Crick pairing rules. The resulting rods self-organize into mesoscale structures.

Immobilization of Molecules to Scanned Probes:

25   There have been some successful efforts to derivatize the surface of a scanning probe microscope probe (or tip) with molecules. One set of methods coats the tip surface with gold and then incubates with organic thiol compound to coat the tip to form some functionalized 30 surface.[38,39,40]

Another set of experiments has relied on the strong non-specific association of bovine serum albumin (derivatized with biotin) and the silicon nitride commonly used to produce such microfabricated tips.[41,42]

35   Similarly, G. Lee et al.[43] have immobilized oligonucleotides to the tips of cantilevers and associated these to complementary oligonucleotides bound to juxtaposed surfaces, and have measured the forces developed as such associations are ruptured by withdrawal of

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said cantilever. Here, oligonucleotides were synthesized to comprise a thiol group which was crosslinked to an amine functionalized organosilane derivatized silica AFM tip. Disruption of association of complementary oligonucleotides was shown to be reversible.

Positional Synthesis of Molecules:

D. Eigler has demonstrate the direct manipulation of atoms adsorbed to metal surfaces with a Scanning Tunneling Microscope, and the direct assembly of molecules from said atoms. This work involved nonspecific interactions between said atoms and said surfaces, and said atoms and the STM tip. These experiments were carried out at liquid helium temperatures under ultra-high vacuum conditions, because these conditions were necessary for the stability of the interactions used and the molecules (multimers of $CsCl_2$) thus produced. These atom-by-atom manipulations provide useful insight into scientifically interesting processes and the properties of otherwise inaccessible compounds, but because of the extremity of the conditions required, do not suggest practical methods for the assembly of useful molecular structures from atoms.

K.E. Drexler[44], after R. Feynman, has made elaborate proposals concerning the positional synthesis of nanoscale, three-dimensional structures, generally on an atom-by-atom basis. Practical methods for such synthesis have yet to be presented, though C. Musgrave has undertaken theoretical analyses of factors related to the assembly of diamondoid structures from reactive carbon species under mechanical or positional control and forces exerted on reactants thereby (referred to as mechanosynthesis).

Objects of the Invention:

It is an object of the present invention to reduce the capital costs of microscale and submicron patterning in the fields of microelectronics, micromechanics and MEMS. It is a further object to reduce the minimum feature size below that attainable with visible light in certain embodiments. It is an object of further embodiments to facilitate the inexpensive prototyping and low-volume production of such devices. It is a yet further object of the present invention to provide for the inexpensive production of miniaturized scanned probe devices. It is a further object of the present invention to provide

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for the used of said miniaturized scanned probe devices in the parallel positional synthesis of complex molecules and supramolecular assemblages.

It is an object of the present invention to extend the
5   applicability of patterning methods to molecular monolayers, multilayers and polymers therefrom.

It is an object of the present invention to provide an inexpensive method for the synthesis of complex copolymer arrays without extensive use of lithography. . It is a further object to extend the methods of
10   use of such complex copolymer arrays to provide enhanced capabilities and refinement of discrimination.

Summary of the Invention:

The present invention employs either contact printing , contact
15   molding, or devices produced thereby, in combination with pattern replication, and applies these to the fabrication of numerous devices. An initial or master relief pattern or pattern of surface composition is produced by prior art methods or by methods of the present invention. This master is replicated one or more times by methods
20   provided within the present invention, permitting rapid expansion of, template size and number of templates . Final generation templates are used in device production. Macroscale to nanoscale features may be achieved by these methods, including within the same fabrication process and article of manufacture. Preferred embodiments include the
25   generation of masking patterns for etch control and diffusion barrier formation in semiconductor fabrication, microfluidics fabrication, MEMS fabrication and the like, patterned chemical synthesis and copolymer synthesis, the formation of patterned molecular monolayers and multilayers, replication of chemical patterns, fabrication of
30   microstructured and nanostructured materials and combinations of any of the foregoing.

Description of the Invention:

Throughout this disclosure, it will be assumed that patterned
35   relief surfaces are composed of materials that are either unreactive to any chemical species which are applied to them or may be pretreated to render them similarly unreactive, or otherwise that any reactivity

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to said chemical species which are applied to said patterned relief
surfaces occurs or may be caused to occur to only a negligible extent.
It is also assumed, as is obvious to those skilled in the relevant
arts, that precautions must be taken to avoid microscopic
5   contaminants, which cause defects at micron and submicron scales; such
precautions include ultrafiltration of solutions, and protection from
atmospheric contaminants. It is further noted that it will be obvious
to those skilled in the relevant arts that adhesion layers, resist
layers and sacrificial layers may be used as needed without departing
10   from the scope of the present invention.

All references are incorporated by citation.

Resist Molding:

As an alternative to the patterning methods described by Whitesides
15   et al. whereby patterns are defined by the transfer an organothiol to
a gold surface and formation of a SAM thereby, a resist or masking
resist pattern may be formed upon a surface by molding an appropriate
material in the desired pattern on said surface or by analogous
techniques such as casting, injection molding, or alternatively by a
20   method comprising the steps of sheet stamping followed by a brief pre-
etch to eliminate protecting material in reduced thickness regions
formed by said sheet stamping step.

In this process, a first relief pattern is replicated in a
convenient material, such as by the polymerization of a prepolymer
25   (preferably an elastiomer) on said surface, casting a plastic,
polymeric or other appropriate solidifiable liquid material with said
first relief surface, plating with one or more metals by known art
techniques, or, stamping into a melted material such as is practiced
in hot-foil technology and CD replication, to define a mold. A mold
30   is thus formed with a pattern defined by said first relief pattern,
and has surface features corresponding to the negative pattern formed
by said first relief (defined as the positive pattern). (Note that
alternatively, said first relief pattern may be fabricated so as to
itself be the negative pattern, from which further negative patterns
35   are generated in even numbered replication generations.) Said first
relief pattern is fabricated either by prior art means or by methods
disclosed within the present invention. Relief feature height (or
depth) is predefined so as to permit reliable pattern formation,

reliable cast or molded material self-cohesion and substrate surface
adhesion, and also reliable release of said cast or molded material
from said from said negative replicated relief. Optimal feature sizes
will vary according to the materials and materials combinations
5   chosen, and specifics of any particular embodiment of this category of
methods.

Said initial pattern may be used repetitively to form the opposite
pattern on a replica surface such that high quality first generation
replicas are formed in a quantity increasing arithmetically with each
10   replication cycle. First generation replicas formed by said first
relief pattern may be used in turn to generate subsequent generation
replicas, with multiplicative increase in replica quantity per
replication generation. Thus tradeoffs will exist between the
quantity of replicas formed in a particular length of time and the
15   quality of replicas thus formed. High fidelity replication methods,
such as are used in the fabrication of integrated optical devices and
components thus permit the rapid production of replicas; negative
replicas are then used to mold a resist pattern onto a surface.

In a preferred case, a prototype master is used repetitively to
20   create negative replicas neighboring each other on a first generation
replica surface, which is then in turn used repetitively to create
replicas of the entire adjacent group of first generation replicas,
again in an adjacent configuration. Thus, a single pattern is
repeated an increasing number of times on the surface generated by
25   each generation of replication. Thus, advantageous tradeoffs between
relief life, rapidity of pattern production, and pattern fidelity may
be attained according to the demands (e.g. time, volume, quality) of
production. Calculations relating these will be obvious from the
necessary empirical data and production constraints.
30   Several different procedures are available for the formation of a
resist pattern on a surface with such a mold. These methods exclude
masking material from regions contacted by the highest extent of said
negative replicated relief, which, if of elastiomeric composition,
will conform to the contours of the substrate surface when juxtaposed
35   to said surface under sufficient normal force. Resist material is not
excluded from recessed regions of said negative replicated relief, and
allowed to harden, polymerize, cure or is exposed to appropriate
illumination to photo-cure, as appropriate, after which said negative

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replicated relief is evenly removed in a manner sufficiently gentle to
ensure that the resulting resist pattern is not damaged.

Note that molded resist layers may favorably be formed on the
surfaces of adhesion layers which have first been deposited or coated
5   onto said substrate surface without departing from the above aspect of
the present invention. Here, for example, an ultra-thin, uniform
adhesion layer which is sensitive to an etching solution or procedure
comprising polymerizable chemical functional groups may first be
situated upon a substrate, which is then, for example, coated
10 uniformly with a photopolymerizable polymeric resist, which is then
patterned under pressure with a clear relief surface and then
photopolymerized by exposure to appropriate wavelength light, with the
resulting polymeric resist material covalently linked to said
polymerizable chemical functional groups of said adhesion layer. Said
15 adhesion layer is chosen to not protect the underlying substrate
surface from etching treatments or procedures, and is formed at a
thickness sufficiently small to prevent the significant under-etch of
the overlying resist material (due to the small cross-sectional area
and correspondingly small diffusion rate through such an area).

20 Note that such an underlayer may, when possessed of sufficient
elasticity, alternatively permit the use of a metal relief replica as
said negative replicated relief because said elasticity serves the
same function as the elasticity of an elastiomeric mold, i.e. ensures
that juxtaposed surfaces subjected to normal forces conform to each
25 other such that liquids do not significantly interpenetrate between
the contacting areas or said juxtaposed surfaces; as above, resist
precursor material is excluded from areas of said adhesion layer
contacted by said negative. In this case, the article being patterned
conforms to the relief rather than the reverse situation with
30 elastiomeric negative replicated relief molds.

A yet further variation involves the use of metal or other rugged
reliefs as "cookie-cutters" which mechanically exclude a softened
resist precursor such as a melt or sol-gel from surface regions
underlying outermost extensions of the applied relief structure.

35

Fabrication by Layered Molding of Patterned Article Material and Sacrificial Material:

Techniques such as those described above for molding of structures using relief masters may be used recursively where each repetition adds another patterned layer (i.e. a portion of a layer in a predetermined pattern which may additionally have surface features or

5   height contours) of material for the desired final article or another patterned layer of sacrificial material useful in the production of unsupported or overhanging structures.

Here, sacrificial materials may include waxes which are melted away at elevated temperatures or dissolved in solvents such as alcohols.

10   These would most favorably be used as sacrificial material with polymers that polymerize in aqueous solution or other solutions in which said wax is substantially insoluble.

Sacrificial material may also be used to flatten intermediate surfaces of article material as an article is being built up by this

15   method. Here, article material is patterned by molding, casting or stamping, and when hardened (if hardening is required,) the article under fabrication is juxtaposingly contacted to a flat surface which is coated with softened or liquefied sacrificial material (where as necessary a release agent is used between said flat surface and said

20   sacrificial material to facilitate removal of said flat surface after said flattened material has set or hardened.) This method is advantageous where an article is conveniently built up in layers with substantially flat borders.

Note that article material is preferably chosen such that where

25   article precursor material (such as a prepolymer) is overlaid onto polymerized or hardened article material, chemical crosslinks will form between these regions of two such juxtaposed layers. This will be the case, for example, for polymers where polymerization occurs via side-groups or where there is an excess of one of the two chemical

30   functionalities which are involved in polymerization, such that a plurality of unreacted functional groups are available on the surface of such article material after polymerization of that portion of article material has proceeded to completion.

Complex mechanical and micromechanical systems with considerable

35   depth and complexity along the depth axis may thus be produced by repetitions of such a procedure, with removal of the sacrificial material used being performed by appropriate treatment after the final layer of article material has been added.

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With this method, prototyping requires the production of initial reliefs corresponding to each pattern of article material, which may be used in turn to pattern such material.

5   **Resist Defect Reduction Methods:**

For microfabrication embodiments of the present invention, variations of the methods of Whitesides et al. are used. The unacceptably high defect densities which have dissuaded these workers from continuing efforts in applying this methods to commercial

10   microfabrication and microelectronics fabrication may be prevented in a number of ways such that an acceptably low defect density may be achieved. By such methods, the simplicity, economy and high resolution capabilities of microcontact printing may favorably compete with deep-UV lithography and other more conventional high resolution

15   patterning techniques.

i. Metal Film Annealing:

The defects encountered by Whitesides and co-workers are attributed by these workers to crystallites arising in the gold film deposition

20   process. It has been determined[45] that an acetylene flame annealing step yields atomically flat terraces extending hundreds of nanometers. STM shows these surfaces to be better coated with an organothiol derivative (dimethylaminoethanethiol) than the unannealed film. M.D. Ward and co-workers[46] have previously found that annealing a gold wire

25   with a hydrogen flame yields atomically flat terraces extending over hundreds of microns. Because atomically flat surfaces are better substrates for the formation of self-assembling monolayers, and because crystallites are reduced or eliminated by such thermal annealing, thermal annealing steps will thus reduce the occurrence of

30   defects when added, before the formation of the patterned organothiol SAM, to the procedure of Whitesides and co-workers. It is, of course, necessary that the annealed film be permitted to cool (i.e. thermally equilibrate with the temperature of the relief and applied solution) before application of the SAM forming material, because thermal

35   contraction will otherwise distort the desired pattern.

ii. Multilayer Vetoing:

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Defects which are small and occur approximately randomly, as is the case as the gold-organothiol resist masking method of Whitesides et al., may be prevented from appearing in the etched final product by a vetoing method which bears analogy to the reticle voting method

5   described in U.S. Patent Number 5,308,722 by J.L. Nistler. An elastiomeric relief may be prepared by related art methods or as otherwise disclosed in the present invention, and then coated with an organothiol solution. In the present case, a thin layer of gold is deposited by suitable means onto a substrate. This is then thoroughly

10   cleaned and contacted with said patterned relief surface which has been coated with an organothiol solution. The pattern of said relief are thus transferred, in the form of a SAM of said organothiol, to the surface of said gold film, in a first printing step. The patterned surface thus produced is then coated again with gold, forming a second

15   gold film layer, and again patterned in a second printing step with the same relief surface coated either with the same or a different organothiol compound solution. The uncoated regions of said second gold film layer are then etched by appropriate solutions, such as strong acids, such that the underlying substrate is exposed in these

20   unprotected regions and thus susceptible to etching and impurity diffusion. Where said substrate is composed of semiconductive materials, electronic, microelectronic, MEMS and other devices may be produced through processes comprising the above method. For defects that occur due to incomplete SAM formation in the contacted regions,

25   whether due to defects arising during the formation of said gold film layers or in the monolayer formation process (i.e. incomplete coverage of a satisfactory gold surface region) which are not due to large surface protrusions, such a multilayer patterning method will prevent defects from leading to the improper exposure of substrate surfaces

30   after said etch step because only defects in the topmost layer organothiol or topmost layer gold film will permit exposure of the next underlying organothiol layer on the next gold layer. Where defect occurrences are independent events from layer to layer, defects are reduced in such a process as the power of the number of layers

35   used; for the above two layer process, the probability of a defect occurring in a unit of area is squared. Assuming a defect 1 square micron in size occurs on average once, randomly, in an area of $1mm^2$, the relative defect area of 10^{-6} yields a defect rate per two-layer

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mask of 10^{-12}, which should be tolerably low for most applications.
Such a defect rate may be reduced further by three or more repetition
of the above coating and printing steps.

Of course, such methods will require correspondingly refined
5   alignment between multiple masking steps, which may be ensured by more
accurately machined alignment means which are designed to fasten to
the reference points (e.g. drilled holes of precise location and
diameter, three or more per different resist used in a pattern
generation procedure) on relief negatives with high positional
10   repeatability. Such considerations and methods for accomplishing the
required alignment will be obvious to those skilled in the arts of
microfabrication. Note that for purposes of the present invention,
reliefs may favorably incorporate features which facilitate alignment
between successively applied relief patterns, such as features,
15   favorably placed at borders which generate Moiré patterns under
appropriate illumination and imaging (which may include electron
microscopy) due to the overlap of patterns in said substrate and said
negative relief. Note further that the methods of the present aspect
of the present invention very directly also permit accurate tactile
20   alignment, where said features comprise interlocking reliefs which
only fit together when said relief and said substrate are in precise
registry with each other. Such tactile alignment methods may thus
permit repeatable accuracy to within a few nanometers with
appropriately designed patterns and sufficiently resilient materials.
25   Higher tolerances will require increasingly careful choice of
materials and control and uniformity of temperature to prevent
significant distortions due to thermal expansion. As will be obvious,
such alignment methods may be used with other aspects and embodiments
of the present invention.
30

iii. Initiator Polymerization:

A further approach to defect reduction is applicable to defects
arising from incomplete SAM formation in relief contacted regions.
Here, the chemical composition of said SAM is chosen such that the
35   exposed surface of said SAM comprises chemical functional groups which
serve as initiators for some polymerizable material. Note that
compounds which do not form SAMs may also be used, provided these are
reliably deposited on said substrate and do not diffuse; compounds

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used need only suitably form patterned initiator compound regions. A
quantity of material of said chemical composition (for example,
dissolved in a first solution) is applied in sufficient but not
excessive quantity to a desired patterned surface, which is then

5   contacted with a substrate to transfer said material of said chemical
composition to said surface in a pattern determined by the pattern of
said patterned surface. Said surface is then washed to remove any
excess reactants or reagents. Said material of said desired chemical
composition, now situated in a patterned manner on said surface, is

10   then contacted with a second solution comprising monomer reactant
species which are capable of reacting with said chemical functional
groups which serve as initiators. Said second solution preferably
contains said monomer reactant species at low concentrations such that
molecules of said monomer reactant species are more likely to react

15   with said chemical functional groups which serve as initiators, or
oligomers or polymers therefrom, rather than with other said molecules
of said monomer reactant species. The limit on the concentration of
said monomer reactant species are those posed by non-specific
polymerization, i.e. significant polymerization of said monomer

20   reactant species into molecules not in communication with said
chemical functional groups which serve as initiators. The term
significant here refers to the quality of the overlayers thus produced
and the non-coating of substrate regions not comprising said chemical
functional group which serves as initiators, i.e. reduction of

25   resolution in non-resist coated regions. These polymerizations are
thus preferably conducted so as to primarily yield solid phase
immobilized polymer products. Said second solution may further
comprise multimeric monomers or other crosslinking reagents which
serve to cause branching of polymer chains during a polymerization

30   process. Conditions of reaction, the concentration of said monomer
reactant species, and the proportion or surface density of said
chemical functional groups are chosen such that the polymer coating
formed will be substantially limited to the regions of said surface
originally contacted by said patterned surface, i.e. not extending far

35   from any of said chemical functional groups which serve as initiators.
For example, monomeric species may be added at a limiting quantity, or
at a concentration sufficiently low that excessive polymerization does
not occur within some conveniently short period of time. This method

of surface coating patterning followed by coating thickening (which
may be termed overlayer formation) serves to enclose any small resist
layer defects without significantly compromising patterning
resolution. Such enclosure reduces or prevents transport of etching

5   agents to defect sites and etching products from said defect sites.
As defect size increases, this method requires that overlayer
thickness be increased correspondingly to ensure enclosure or
encapsulation of defects of said defect size.

      For example, said chemical composition of said SAM may be chosen to

10 comprise an alkanethiol moiety and a methacrylate moiety. A solution
comprising a quantity of said chemical composition is applied to a
gold film situated on a substrate, such that a SAM is formed according
to the pattern of said patterned surface. Said gold film and
substrate is then washed to remove any excess unbound material.

15 Limiting quantities of methacrylate and any desired polymerization
accelerators, as well as a desired ration of polyfunctional or
branched methacrylate species are added, and overlayer polymerization
is permitted to occur to a desired extent, corresponding to the defect
size to be thus eliminated. Unreacted materials are then washed away

20 with appropriate solvents that do not degrade said overlayer. The
surface thus treated is thus patterned with a resist layer of
corresponding thickness which does not have pinhole defects or other
small defects smaller than some critical size.

      Note that the methods of this aspect of the present invention are

25 readily applied to patterned thin film coatings which are not SAM
based, provided that appropriate chemical functional groups which
serve as initiators for some polymerizable material may be
incorporated. Note further that any chemical functionality which may
be modified (e.g. deprotected, activated, or reacted with some other

30 initiator bearing chemical species) such that it may later serve as an
initiator for polymerization reactions is comprehended within the
spirit of this aspect of the present invention.

Resist Formation by Wettability Control:

35   The methods of G.M. Whitesides and co-workers involving the wetting
of patterned surface regions, which these workers have used to form
microlens arrays from polymer solutions, may also be applied to the
elimination of small defects. In this approach, patterns of resist

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are formed by first patterning a SAM which is preferentially wet by a
resist monomer solution or precursor solution on a surface which is
poorly wet by said solution. Said patterning is accomplished by one
or more of the methods described herein, such as microcontact

5   printing, and the patterned said surface is then contacted with a
resist precursor solution, which is then withdrawn. Portions of said
resist precursor solution retained on said surface are then caused or
permitted to polymerize, such that a developed resist layer is formed.
Thus, defects related to incomplete masking may be eliminated by their

10   enclosure by the overlying resist precursor solution and hence the
resulting developed resist. Etch steps are then performed as in
conventional microfabrication practice.

In the case of pattern which cause self-organization of said resist
precursor solution, and hence predetermined desired geometries of said

15   developed resist layer three dimensional structure, resist depth-
dependent etching, in analogy to that described by G. Gal in U.S.
Patent Number 5,310,623, may be performed, for example by reactive ion
etching, to replicate the self-assembled resist structure in the
underlying solid substrate material.

20

Patterned Metallization:

Metallization, for example, for the formation of electrical
contacts, may be accomplished according to the patterning methods of
the present invention and corresponding extensions of related art

25   methods, as well as by the application of related art methods to
articles produced by the methods of the present invention (e.g.
formation of alkanethiol patterns by microcontact printing on gold
films, employing, as necessary, the defect reduction methods disclosed
herein).

30   Molded materials such as those used above for resist purposes may,
with appropriate solvents, be used as lift-off layers which eliminate
any metallic film situated on the surface of said molded materials
upon exposure of the surface comprising these to said appropriate
solvent. Patterns of said metallic film remain in regions which were

35   not covered by said molded materials.

As will be obvious, formation of metal films may be accomplished by
vacuum evaporation of the corresponding metallic material onto the
surface of the article under production. Alternatively, such lift-off

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patterning may be applied to thin electroformed (e.g. electrodeposited or electroless plated) metallization layers.

Alternatively, metallization may be accomplished by the hot embossing of a first surface with a metallized polymeric foil with the
5   metallized surface juxtaposed to said first surface, under sufficient pressure and at sufficient temperature to ensure good electrical contact with exposed regions of said first surface, followed by dissolution of said polymeric foil and underlying lift-off regions. Such transferred films may be subjected to brief electrodeposition
10   steps to ensure good electrical contact, though care.must be taken to avoid the formation of short circuits.

Microelectronics Device Fabrication:

The foregoing fabrication methods comprising the use of relief
15   patterns used to pattern resist or masking layers may be used to control the etching and doping (impurities diffusion) of semiconductor materials and overlying dielectric layers, as well as the formation of metallization layers for electrical interconnection. These methods serve all of the patterning functions required in microelectronics
20   fabrication.

Note further that the above described resist molding and casting methods may similarly be applied to conductive polymers compositions such as those comprising polyparaphenylene and derivatives thereof, polypyrrole and derivatives thereof, polythiophene and derivatives
25   thereof, or other such conducting or electroactive polymers, so as to directly form polymeric devices rather than resist patterns. These materials and devices based thereupon may further be combined with more conventional microelectronic devices fabricated by the methods of the present invention.
30   Initial relief patterns may be formed in, for example, glass or silicon surfaces by known art methods including photolithography, or may be formed by appropriate scanning probe based patterning methods of related art, or combinations thereof. For example, a glass surface may be coated with a gold film, upon which an organothiol SAM is
35   formed, which is then patterned by STM or field emission mode STM, followed by an acid etch to remove exposed gold regions and a base etch to remove thus exposed glass regions.

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It is important to note that a large patterned area may be formed
by the replication of a smaller pattern from an initial relief across
said large patterned area, as, for example, a single integrated
electronic device pattern layer (e.g. corresponding to a mask layer)

5   repeated many times across the surface area corresponding to a wafer
diameter. Thus, the replication methods of the present invention may
be applied both to expand the number of occurrences of a pattern on a
single surface as well as the number of surfaces thus patterned.

10   **Spatially Controlled Copolymer Synthesis:**

A relief patterned surface, preferably of elastiomeric composition,
may be used to spatially control the pattern of addition of monomer or
macromonomer reactants to a subset of appropriate chemical reactive
sites on a substrate surface. Here, instead of the addition of single

15   species to surface regions according to the pattern of an elastiomer
surface, as done by Whitesides et al., reactants are added to regions
of the surface of said substrate according to the pattern of said
patterned surface, permitted or caused to react, and unreacted or
unbound species are then washed away. Generally, said substrate will

20   comprise one or more substantially flat surface regions and be
prepared by the reaction or adsorption of some chemical species to
said surface such that chemical species having desired reactivities
are bound to said substrate, which is thus capable of being reacted
with desired reactant species. Said relief patterned surface is

25   partially or completely coated with a solution of a desired reactant,
or said desired reactant is deposited from vapor phase onto said
relief patterned surface. In the former case of reactants dissolved
in said a solution, said a solution may further comprise inert
compounds which increase the viscosity of said solutions. Said relief

30   patterned surface is then contacted, in a contacting step, with said
substrate to transfer said desired reactant to one or more
predetermined regions of said substrate. Thus, said desired reactant
is applied only to the desired regions, according to the predetermined
pattern of said relief patterned surface.

35   The control of precise copolymer sequence thus synthesized is
favorably achieved, for example, through the use of
protection/deprotection or activation chemistries to effect step
control over reactant addition to the monomer or copolymer species

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with which said desired reactants are reacted. Thus, reactants added
will generally comprise a protecting group or an activatable reactive
group, which serve to prevent multiple monomer or macromonomer
addition to the same molecule. Through successive cycles comprising
5   the steps of contacting said substrate with desired patterned surfaces
coated with desired reactant species, to permit spatial control over
reactant addition, followed by substrate washing steps, and steps
preparing product copolymer species for subsequent reactions (e.g.
deprotection steps or activation steps by chemical or physical
10   treatments of said substrate surface according to the polymerization
chemistries and step control chemistries employed), complete spatial
control over synthesized copolymer sequence may be achieved. During
successive cycles, different patterned relief surfaces are used at
different steps, and may optionally be shifted in location from one
15   step to the next; similarly, different desired reactant species may be
used during different cycles. This is in analogy to multiple masking
steps in related art microfabrication, and directly related to the
multiple masking methods of other aspects of the present invention.

By such methods, arrays of copolymers of predetermined sequence,
20   such as oligonucleotides, polypeptides, biomimetic copolymers and non-
biological copolymers may be produced at a resolution equal to that
with which a relief master may be fabricated. Patterns having
features many times smaller than one micron are thus achievable, and
correspondingly high densities (such as 10^8 to 10^{10} array elements per
25   cm^2) may be achieved without extensive use of costly lithographic
equipment. Such methods thus carry several advantages over the
methods of S.P.A. Fodor et al. in that protection/deprotection
chemistries (and the corresponding reactant compounds or
protected/deactivated) need not be limited to those responsive to
30   actinic radiation or other energetic beams. In other words, reactants
useful for ordinary (e.g. not light controlled) stepwise syntheses are
useful with these methods. Further, these methods are suitable for
the synthesis of copolymer of compositions which might be or are
desired to be sensitive to photochemical or other energy induced
35   degradation.

In a second variation, such a said patterned surface may be used to
control the spatially controlled activation or deprotection of
copolymers or copolymer precursors, such that only the copolymers or

copolymer precursors located at predetermined regions of said surface
are prepared for subsequent reaction with a monomer or macromonomer,
which is thereby added only to said copolymers or copolymer precursors
located at said predetermined regions. Here, a relief is for example

5   coated with solutions comprising deprotection or activation reagents
at sufficient concentrations (and preferably also inert constituents
increasing the viscosity of said solutions) and contacted with said
surface to effect the spatially controlled deprotection of the product
precursor copolymers situated thereupon. A solution comprising said

10   monomer or macromonomer is then contacted with said substrate such
that these species may react with any available corresponding reactive
groups.

The methods of this aspect of the present invention thus permit the
synthesis of a potentially large array of diverse copolymer species

15   situated on a surface according to a predetermined pattern, such that
composition of the copolymers at a particular region of said surface
correspond in a known fashion to the location of said region. Thus,
where activities are uniquely displayed by particular regions of said
surface, the copolymer sequence composition responsible for said

20   activities may directly be deduced from information about the spatial
location of said particular regions.

Note that this method may be used to synthesize or otherwise target
copolymers, affinity groups or other molecular functionalities or
decorations to predefined regions of the surfaces of microreliefs,

25   micromolded components and components of micromechanical or
microelectromechanical systems. In these instances, the reliefs used
for pattern formation are produced from master structures which are
identical to the molded surfaces in regions to which said reliefs are
to contact said microreliefs, said micromolded components, etc., and

30   thus situate reactant monomers or other reactive compounds, but which
have surfaces or surface features of increased height at regions not
to be targeted by said reactant monomers or other reactive compounds.
In other words, the reliefs used to target reactants to predetermined
regions of contoured surfaces conform to the geometry of those

35   surfaces in regions to be targeted or modified, but do not contact
regions to not be affected due to clearance within said relief that
prevents contact of the surface of said relief with regions of said
contoured surfaces not targeted.

In the case of copolymer synthesis on preferably flat surfaces, reliefs may be mounted on a roller apparatus, in analogy to arrangements used in holograph stamping processes, to facilitate rapid patterned deposition of reactants.

5   Note that for variations relying on selective contacting of said substrate with reactants rather than with deprotection reagents, rinse steps may favorably be performed with reagents that react with unreacted monomer reactants to inactivate said unreacted reactants such that these will not, upon washing, react with species bound to

10   regions onto which said unreacted reactants were not contacted by said relief. For example, in the case of phosphoramidite oligonucleotide synthesis chemistries, alcoholic solutions may be used to inactivate and wash away unreacted phosphoramidite monomers, which will react with the hydroxyls of alcohol molecules.

15

Example Synthesis Procedure:

A first surface, such as a glass surface, is patterned with a first alkylsilane compound comprising an extended methylene or polyethylene glycol linker, or other substantially linear linker chosen to be well

20   solvated or wetted by oligonucleotide synthesis solvents or chosen to be readily modified so as to be well solvated or wetted by oligonucleotide synthesis solvent, and an esterified terminal hydroxyl group by use of an elastiomeric relief with a square grid pattern such that square regions of said first surface are modified by said first

25   alkylsilane compound with distinct unmodified borders (which are hydroxylated) remaining. Untreated regions of said surface may then optionally be permitted to react with a second alkylsilane compound comprising a terminal functionality which will be poorly wetted by the solvents containing oligonucleotide synthesis reactant monomers. Said

30   first surface is then treated with appropriate reagents such as bases to hydrolyze the terminal ester group of said first alkylsilane and thus deprotect the terminal hydroxyl group. A solution of 3' or 5' protected oligonucleotide synthesis reactant monomers of one nucleobase type is then coated onto a second surface, onto which an

35   elastiomeric relief is impressed to transfer some of said reactant to the surface of high regions of said elastiomeric relief (with raised square regions corresponding to a subset of the raised square regions of the relief used to form the regular array of said first

alkylsilane), or the entire surface of said elastiomeric relief is
directly coated with a solution of said reactant monomer. Said relief
with reactant situated on its surface is then contacted, in an
orientation and position which aligns the array element features of

5   said relief with those of the previously formed grid of the
alkylsilane on said first surface, with said first surface, such that
said reactant is contacted with the exposed hydroxyls of said first
alkylsilane compound, and reactions occur linking this nucleotide
monomer with the hydroxyls of said first alkylsilane compounds in the

10   affected array elements. Such a process is carried out with different
relief patterns and the remaining three nucleotide monomers, such that
all array elements experience the positionally controlled addition of
exactly one of the four nucleotide precursor species. Note that in
this example, said first surface regions which may have optionally

15   been treated to be poorly wetted by said reactant solutions will serve
as borders impeding the spreading of these reactants beyond targeted
array elements; said well solvated linker moieties are drawn into the
solutions with which they are contacted, even where these are situated
on surfaces which are poorly wetted by said solutions with which they

20   are contacted (in which case undesirable spreading of reactants to
inappropriate surface regions is prevented while synthesis is not
frustrated). Said first surface is then thoroughly rinsed. Capping
reagents (such as acylating reagents) may optionally be applied to all
of said first surface to prevent any further elongation of unreacted

25   hydroxyl groups. Other standard oligonucleotide synthesis steps, such
as oxidation, may then be performed as required by the particular
chemistry used. The entire array is then treated with reagents to
deprotect the reactant monomers added during the previous cycle (e.g.
submerged in a solution comprising trichloroacetic acid in the case of

30   trityl protecting groups, exposing a terminal hydroxyl group which is
the target for further reactants). The second base of all array
elements is then added, in a nucleotide addition cycle comprising the
coating of four distinct reliefs (for example, together selecting all
array elements but sharing none) with four distinct reactants, and

35   four contacting steps as above, followed by appropriate steps
according to the chemistries used, as above. Such cycles are repeated
until the array thus synthesized substantially consists of
oligonucleotides of the desired length (i.e. n cycles total to produce

an array of n-mers) and desired element sequence according to position
within said array. Finally, steps deprotecting nucleobase moieties
and, where applicable, deprotecting the phosphate backbone, are
performed to yield the desired final molecular structure, as in
5   conventional oligonucleotide synthesis methods, excepting support
removal steps. Information regarding the configuration of raised
array elements, the reactant and cycle with which they are associated,
is preserved such that position of an array element corresponds in a
known way with the sequence of reliefs and reactants used to
10 synthesize the oligonucleotides therein, which in turn thus have the
corresponding sequence composition. The density (number of array
elements and hence distinct sequences per unit area) of arrays thus
synthesized may rival or exceed that possible with light directed
patterning methods. An important advantage of this method is that
15 conventional oligonucleotide synthesis reagents may be used.

Alternative copolymer array patterned synthesis methods:

The relief forming methods described above may be used to produce a
20 stencil capable of masking surface regions from exposure to reactants.
Here, a substrate is first coated uniformly with a sacrificial layer,
which is hardened or set. Then, an elastiomer precursor is applied to
the surface of said sacrificial layer and then molded by a negative
relief. Alternatively, analogous casting or injection molding may be
25 utilized, or embossing of a polymeric film with brief post-etching of
the embossed film may be used. Said sacrificial layer is then
dissolved to free the patterned elastiomeric stencil.

Such a stencil is used in combination with an array of
microfabricated columns, said columns being of uniform height, and
30 with a pitch smaller than the smallest stencil feature. Said stencil
may either be combined with said array of microfabricated columns into
one stenciling body, or said stencil and said columns may merely be
fused together. In either case, said stencil is juxtaposed to the
substrate which is to be subjected to patterned copolymer synthesis,
35 and said array of microfabricated columns is pressed onto said
stencil, such that said columns force the stencil surface into good
masking contact with said substrate. Solutions containing reactants
(e.g. deprotection reagents or monomer reactants) are then caused to

flow through said array of microfabricated columns, such that areas
not masked by said stencil are exposed to said solutions. Before said
stencil is released from said substrate, thorough wash steps are
performed to remove residual reactants or reagents. Differently

5   patterned stencils are used in predetermines sequence with different
monomer types to produce the desired diverse copolymer array with
sequence correlated to location in a predetermined manner.

In the case that said array microfabricated columns is to form one
body with said stencil, these may be joined together, e.g. by coating

10   the upper surface of said stencil with an adhesive or crosslinking
agent which will react with both the material of said columns and the
material of said stencil. Compounds used for such joining must be
selected so as to be inert to the reagents and solvents which will be
passed over them.

15   Note that said array of microfabricated columns may favorably be
produced by LIGA methods or other replication methods including those
of the present invention, and may favorably be composed of metals,
e.g. formed by electrodeposition or electroless plating methods.

20   <u>Uses of copolymer array decorated articles:</u>

Surfaces prepared according to the above aspect of the present
invention may be used in a variety of ways including for sequencing by
hybridization (where said copolymers are oligonucleotides, or
oligonucleotide analogs), sequence confirmation by hybridization, for

25   the screening of small peptides and the determination of affinity
interactions (as described by Fodor et al., referenced elsewhere
herein), for the detection or determination of antibodies, in clinical
medical assays, and in the screening of organic material for desired
properties according to known methods in combinatorial chemistry.

30   Sequencing by hybridization, as well as other techniques involving
binding determination, may favorably be performed by contacting
samples with such an array, where sample molecules binding to said
array is monitored by any of se: ral known art techniques, while
physical or chemical conditions are changed and binding is recorded

35   (e.g. by capture of signals detected by such monitoring and recorded
electronically) as a function of conditions. In a preferred instance
of binding determination with variation of physical and/or chemical
conditions, the condition dependent properties of sets of array

elements (i.e. copolymer sequences) of interest are determined by
observation of condition dependent binding of known samples, and
information thus obtained is used to further refine the discrimination
of correct (i.e. most highly matched) or most specific binding from

5   incorrect or relaxed specificity binding.

For example, in this way, difficulties in sequencing by
hybridization methods arising from differences in the conditions for
optimal binding specificity which correspond to individual array
elements (oligonucleotides), which frustrate attempts to find globally

10   optimal binding conditions without sacrificing the specificity of many
or some of the array elements in an array comprising large numbers of
distinct oligonucleotide elements, may be overcome. Here, conditions
which may be varied include temperature, ionic strength, divalent
cation concentration, presence and/or concentration of

15   tetramethylamonium chloride, presence and/or concentration of
denaturant compounds such as formamide or dimethylformamide, pH, and
other factors known to affect the hybridization specificity and
stringency of oligonucleotides or polynucleotides, may be varied
individually or in combination. For example, the binding, and also

20   dissociation of a particular sequence with the complementary n-mer
oligonucleotide will occur at a characteristic temperature (i.e.
having a characteristic T_m melting temperature at which half of all
homoduplex complexes dissociate), and this property verifies the
correctness of base pairing.

25

Array Detection of Rare Molecular Species:

Such arrays may be used to detect very low quantities, approaching
single molecules, of complementary molecules in samples by a few
distinct methods.

30   According to the first method, a target array is designed in a
hierarchial manner such that as monomers or subunits are added, they
are added to subsets of regions to which the previous monomers or
subunits were added. For example, an oligonucleotide array is produce
by forming first square regions, subdivided into four square regions

35   each containing one of adenosine, guanosine, cytosine or thymidine
nucleotides (e.g. through a set of four steps, each step adding one
nucleotide to one subdivision region). During the successive
synthesis steps, identical squares with sides one half that of those

of the preceding step and area one quarter that of the preceding step
are formed, again subdivided into four square regions each containing
one of the four nucleotides, preferably in the same relative
configuration as that of the preceding set of steps, are added, such
5   that one subdivision square of the previous step is thus subdivided
into four subdivisions. Such a hierarchial subdivision process
comprising n repetitions of the above sets of four steps (one for each
nucleotide moiety) is repeated to produce an array of 4^n n-mers, or
desired subsets thereof. For purposes of detecting a rare molecular
10   species, a set of arrays of oligonucleotides of increasing length,
e.g. {(n-m), (n-m+1)...(n-1), n}-mers in the case of single nucleotide
increments, is produced as above. This set of arrays is designed such
that each successive array in the series is in the mirror image of the
preceding array of said series, with at least one further synthesis
15   cycle (set of synthesis steps) further subdividing the regions
opposed to the smallest array elements of the preceding array.
According to the expected relative abundance of the species in a
sample to be detected, said sample is bound first to the array
consisting of the smallest number of elements. Thus the sample is
20   divided such that each oligonucleotide targets a region of some subset
of molecules in said sample. Hybridization to such a lower density
array by rare species will be more accurate under appropriate
conditions in that fewer array elements of larger area present more
molecular targets and fewer degrees of freedom. Thus the kinetic
25   limitation on binding depends mainly on the diffusional transit time
from across said array. After a sample is bound to such a lower
density first array, it is juxtaposed to a further subdivided mirror
image second array, with only a small gap between these arrays, which
is filled with an appropriate buffer. Conditions are changed to
30   denature the association of sample molecules with said first array,
and then to binding conditions sufficiently stringent that said sample
molecules will favorably bind to their target oligonucleotides on said
second array in preference to forming the lower energy association
with their shorter targets on said first array. This process results
35   in successive subdivision of said sample, such that each subdivision
step successively narrows the volume which must be traversed by a
sample molecule in order to find its correct binding target, and

further correspondingly reduces the chances that a molecule will find an incorrect target.

An analogous method within the scope of this first method may be applied where an array is designed, as above, such that array elements

5   are similar (but not identical) in sequence to most of their nearest neighbors, such that spatial proximity is related to ordered sequence similarity. This similarity may be used to serve a similar "narrowing down" function accomplished above by juxtaposition of hierarchially related mirror image arrays. In the present case, conditions

10   affecting binding stringency are varied such that after a sample is first applied, only a few contiguous base pairs are sufficient for binding. The regions bound by a sample molecule comprising a specific sequence may either be within the contiguous region comprising many array elements having m of the total n monomers in common (starting

15   from the first variable monomer added during the first cycle of array synthesis), or with m contiguous monomers offset from the copolymer terminal from which synthesis began, in which case, for each class of array elements with said m monomers in common, contiguous regions of elements comprising said m monomer sequence complementary to said

20   specific sequence, where classes are distinguished according to the number of monomers by which said m monomer sequence is offset from said first variable monomer, become increasingly small and increasingly distant from each other. This results in reduced dwell time in incorrect regions (incorrect with respect to the exactly

25   complementary n-mer target) but in the presentation of larger contiguous targets by regions comprising the n-mer target. In other words, said specific sequence binds to the correct region comprising the n-mer target and similar n-mer sequences according to an equilibrium expression where the proportion of said specific sequences

30   bound to correct regions is proportionate to the concentration of unbound said specific sequences times the relative area of said correct region (in direct analogy to the concentration of m-mers in a conventional equilibrium constant expression). Conditions are then made gradually more stringent, such that more associations between

35   contiguous base pairs are demanded for binding. Stringency (i.e. the conditions affecting stringency) may be oscillated in an increasing trend, such that sample molecules are most likely to home in on their final target oligonucleotides. Those sample molecules which do not

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initially bind sufficiently near their targets will enjoy increased
diffusional mobility as those sample molecules which have bound to
said array are less likely to impede their diffusional transport
through the sample solution, e.g. after they no longer bind m-mer
5   comprising elements further from their correct targets under more
stringent conditions.

Generalizing this method to any copolymer with sequence dependent
binding properties, physical and chemical conditions may be varied to
examine the characteristics of, or detect the presence of, binding
10   activities corresponding to respective sets of conditions, or spatial
or temporal gradients thereof.

Note that designations above of terminal m-mers are chosen for
clarity rather than limitation. The essence of the forgoing
description concerns the hierarchially imposed proximity of array
15   elements with similarity which generally decreases with increasing
distance of elements in said array. While an m-mer may occur in many
array elements in many distinct regions of said array, it remains the
case that the m-mers in phase with the target n-mer sequence are
grouped together in one contiguous region comprising said target n-
20   mer, regardless of the ordering of synthesis cycles with respect to
such hierarchiality.

A second method, applicable to polynucleotide samples, involves the
amplification of rare species, or sequence fragments thereof. In this
case, established art methods such as the polymerase chain reaction
25   (e.g. with PCR primers flanking the diverse target sequence) or the
ligase chain reaction (e.g. for an 8-mer array, involving all 4-mer
oligos under conditions sufficiently stringent to preclude any
mismatch within said 4-mer sequences for binding) are used to amplify
the sample target sequences, which may be a diverse sequence in a
30   conserved sequence context. Thus, small quantities of molecular
species within heterogeneous samples may be amplified to a sufficient
extent that detection of binding to an oligonucleotide array is
conveniently accomplished. Amplification reagents (e.g. primers or
nucleotides for PCR or oligonucleotides for LCR) may be labeled, for
35   example, with one or more affinity moieties (e.g. biotin, digoxigenin,
etc.,) or one or more fluorescent dye moieties (e.g. Texas red,
rhodamine, fluoroescien, etc.,) or one or more other reporter groups
or portions. For these amplification methods, amplification will

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produce a geometrically increasing quantity of sequences matching the
amplified sequences but also a related quantity (which may be biased
by such known art methods as asymmetric PCR)

5   **Spatially Controlled Macromolecular
        Collocalization:**

        Similar operations as used to synthesize copolymers on a surface in
        a spatially predetermined manner described above may similarly be used
        to collocalize multiple distinct macromolecule or macromolecule types
10   to predetermined surface regions. For example, such methods may be
        used to generate a very large number of positionally predetermined
        combinations, shapes and/or patterns of large numbers of adhesion
        proteins which may be used , for example, to test the effect on cell
        adhesion, cell motility, cell shape or cell physiology in biological
15   and clinical assays, as shown by Whitesides and co-workers for
        patterns consisting of a single protein species, as well as in other
        biotechnological uses, including the interfacing of cells to
        microelectronic devices and sensors. These methods may avail the
        protein patterning methods disclosed by Whitesides and co-
20   workers[47,48], or may avail other immobilization chemistries. The
        combination of protein patterning with combinatorial methods thus
        enabled permits the study of competing influences when different
        pattern, relative abundances, and different combinations coexist.
        Note that in these cases, the binding of the macromolecules, which may
25   or may not be macromonomers, may be according to affinity interaction
        or less specific weak interactions, i.e. need not be covalent binding.
        The present aspect of this invention thus depends on the use of a
        relief pattern comprising raised surface elements to contact a
        solution containing one or more protein or polypeptide to portions of
30   a first surface to which said protein or polypeptide will adsorb,
        wherein contact only occurs between said raised surface elements of
        said relief pattern and said first surface, such that a pattern of
        said protein or polypeptide is deposited in the pattern corresponding
        to said raised surface elements onto said first surface, wherein such
35   patterning steps are repeated to yield deposits of plural different
        proteins or polypeptides at different regions of said first surface,
        such that different combinations result in a spatially predetermined
        manner.

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Patterned Combinatorial Materials Deposition:

As reviewed above, X.-D. Xiang et al.[49] have recently described a
method for the production of arrays of combinations of material, which
are then screened to discover combinations with useful properties.
The patterning methods described herein may be extended to the
production of such arrays but further permitting the rapid, selective
deposition of materials which are not conveniently evaporated or
without the use of evaporation equipment. Here, a suspension of very
fine particles of a material is applied to the surface of a wettable
relief, which is preferably of elastiomeric composition. The liquid
layer of said suspension on said relief is sufficiently thick that an
adequate quantity of suspended material per unit area is obtained on
the surface of said relief. This will depend on the viscosity and
surface energy of the liquid used. Such a suspension coated relief is
then juxtaposingly contacted to a porous flat surface, which may be
the surface of a filter. Depending on the composition of the material
of said flat surface, capillary action may draw the particles in said
suspension onto said flat surface, as in slip-casting. Material
transfer of this kind will only occur in areas of contact with said
relief, i.e. those raised areas of said relief. Where the composition
of the material of said flat surface does not suitably permit
capillary action to be relied upon with the solution used to suspend a
material, said flat surface must be that of a porous filter, so that
negative pressure may be used to draw the suspension solution through
said filter, depositing suspended material onto said filter surface.
After repeated cycles of deposition of at least two but preferably
more different suspensions of different materials, in a pattern
determined by the patterned relief used, a patterned array of
different materials mixtures is yielded. This may then, for example,
be subjected to annealing steps. Thus, large numbers of different
combinations of materials may be produced in a convenient format for
testing and characterization, including from materials which are not
suitably evaporated.

Template Patterned Synthesis of Sheet-like Complexes and Copolymers:

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The formation of chemical patterns on surfaces permits the use of such chemically patterned surfaces as templates for the production of patterned molecular complexes and copolymers, which themselves may be used as templates for complementarily patterned molecular complexes

5   and copolymers. This aspect of the present invention is analogous with other aspects of the present invention in that spatially patterned surfaces may be replicated from an initial pattern and thereafter from each other. In the present aspect, patterned compositions of sheet-like molecules serve as *molecular* templates for

10   the definition of patterns in thus complementarily patterned sheet-like molecules in a molecular replication process analogous to the polymerase chain reaction invented by K. Mullis for the amplification of polynucleotide molecules.

The foregoing pattern replication methods of the present invention

15   and those found in related art, as well as scanning probe based patterning methods[50,51,52] may be used to perform the patterned chemical derivatization of surfaces. As shown by examples from related art, such modifications may cause the preferential binding of molecular or macromolecular species to predetermined regions of said

20   surfaces, which are demarcated by said patterned chemical derivatization. Such surface modifications may be used, for example with the methods of G.M. Whitesides et. al. or those of W.T. Müller, P.G. Schultz, et al., to bind specific first affinity groups or species to predetermined regions of said surface. Said affinity

25   groups or species are chosen so as to bind affinity derivatized layer forming molecules comprising a second set of affinity groups or species which are in communication with a layer forming precursor moiety (such as a polymethylene chain, or rigid rod oligomer or polymer). The affinity moiety of said second set of affinity groups

30   is preferably located at one terminus of said affinity derivatized layer forming molecules, which are preferably substantially linear in structure. Said affinity derivatized layer forming molecules preferably but not necessarily further comprise a moiety of third affinity group or species, which is preferably located at the terminus

35   opposite that at which said affinity moiety of said second set of affinity groups is located; said affinity derivatized layer forming molecules may also comprise one or more (preferably two or more) distinct reactive, cross-linkable or photopolymerizable chemical

functional groups, situated along said layer forming molecules such
that each of said distinct reactive, cross-linkable or
photopolymerizable chemical functional groups occurs at a particular
position along the length of said layer forming molecules, and may,

5   after a layered structure has been formed by association of moieties
selected from said second set of affinity groups with said first
affinity groups pattered on said surface, be caused to react to form a
covalently linked network joining said layer forming molecules. Said
first affinity groups and said second affinity groups are preferably

10   chosen such that the interaction between them is sufficiently strong
and specific under a first set of conditions but is substantially
weakened or neutralized under a second set of conditions; thus, under
said first said of conditions, layer forming molecules assemble at
surfaces comprising regions of said first affinity group in patterns

15   corresponding to the pattern of said regions, while said second set of
conditions serves to separate layers comprising said affinity groups
chosen from among said second set of affinity groups from surfaces
(including surfaces of layers) comprising said first affinity groups.
Said third affinity group or species is chosen such that it does not

20   bind to either of said first affinity groups or said second set of
affinity groups, but will bind to affinity groups selected from a
fourth. set of affinity groups, which in turn will not bind to said
first affinity groups, other affinity groups selected from said fourth
set of affinity groups, nor to said second affinity groups (i.e. said

25   first affinity groups and affinity groups of said second set of
affinity groups bind only to each other and not to identical affinity
groups; said affinity groups chosen from said third set of affinity
groups and said affinity groups chosen from said fourth set of
affinity groups bind only to each other and not to themselves; e.g.

30   sets of affinity groups may each consist of one of adenine, thymine,
cytosine or guanosine nucleobases). The composition of said surface
in unpatterned (or differently derivatized regions) is chosen to not
bind said affinity derivatized layer forming molecules. After a
pattern of said first affinity groups is formed on said surface, a

35   solution containing said affinity derivatized layer forming molecules
is contacted with said surface under appropriate conditions and for
sufficient time to permit the binding of said affinity derivatized
layer forming molecules to all available sites on said surface.

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Unbound species are washed from said surface. Thus, said layer
forming molecules comprising affinity groups from said second set of
affinity groups are bound to said surface in a pattern replicating the
pattern of said first affinity groups on said surface. These may then
5   be cross-linked via said reactive groups, for example by the addition
of suitable cross-linking agents, photopolymerized, or caused to react
together by establishing appropriate physical or chemical conditions
such that a covalent network is formed. Note that said affinity group
chosen from said third set of affinity groups may be initially caged
10   or protected to suppress the formation of associations until a
deprotection step is performed. In this instance, affinity groups
chosen from said fourth set of affinity group may be reacted with
regions of said surface not containing said first affinity group, in
direct analogy to the complementarily patterned two component
15   monolayers disclosed by G.M. Whitesides and co-workers, formed by
patterning a first component monolayer and then permitting a second
component compound to self-assemble into a monolayer on remaining
uncoated regions. Alternatively, patterns composed of domains of
multiple distinct affinity moieties may be formed by multiple relief
20   contacting steps, or by multiple spatially selective activation steps
(examples include: light directed, for photodeprotectable or
photoactivatable compound derivated surfaces; scanning probe
controlled surface catalysis such as the methods of W.T. Müller, P.G.
Schultz et al., or of B.J. McIntyre, M. Salmeron and G.A. Somorjai, or
25   other scanning probe based patterning methods capable of directing the
adsorption or reaction of molecules comprising the desired affinity
moieties to the selected regions of patterned surfaces; and, the
replication methods of related art or of the present invention, e.g.
involving microcontact printing). Multiple distinct molecular species
30   comprising multiple distinct affinity moiety types or classes and
types may be patterned onto the same surface by repetitive cycles of
patterned activation or patterned masking followed by deposition of
the respective compound (e.g. by contacting a solution of said
respective compound to said patterned activated or patterned masked
35   surface) such that deposition occurs at all available sites. Thus, a
first pattern may be formed, and the regions thus specified caused to
react with a first chemical compound by contacting said surface with
said first chemical compound or solutions thereof, a second pattern

may be formed and the regions thus specified caused to react with a
second chemical compound by contacting said surface with said second
chemical compound or solutions thereof, and so on, until patterns of
all desired patterns of all desired chemical compounds (comprising the
5   corresponding distinct affinity moieties) are formed.

After the polymerization of such a patterned layer into a sheet-
like polymer, it is released from the initial patterned surface, e.g.
by exposure to elevated temperatures. The affinity groups on each
side of said sheet-like polymer may then be used, by similar steps as
10   those used to produce said sheet-like polymer, as a template for the
formation of complementarily patterned sheet-like polymers by exposure
to appropriate affinity group terminally functionalized layer forming
monomers. Repetition of such layer forming steps thus provides
geometric amplification (here for fabrication purposes) of the
15   patterned sheet-like polymer, in analogy to PCR, provided that the
sheet-like polymer produced at each step is freed, as above, from the
template surface which directed its formation.

Patterns thus generated may be designed to facilitate orderly
binding together of such layers under appropriate conditions (e.g.
20   thermal annealing) into bi-layer or multilayer structures, in analogy
to the hybridization of DNA molecules. Such design will favorably
include pattern elements that ensure proper sheet-like alignment with
proper registry before bulk or random associations between surface
regions of said layers are permitted to occur.

25   For example, considering a square sheet-like structure, at
sufficiently low concentration to preclude significant trimolecular or
tri-complex reactions, one corner of each of two sheet-like structures
to be juxtaposed may comprise thiol groups, which thus target these
corners to each other before affinity interactions are permitted to
30   occur. A second set of corners, diagonally located from said first
set, may comprise primary hydroxyls, which are then crosslinked by an
appropriate crosslinking agent, such as a bifunctional acid anhydride
compound. Thus, a first set of corners are targeted together,
followed by the diagonally opposite set, after which registry of
35   appropriately designed affinity patterns causes registry of the
remainder of the juxtaposed surfaces.

Where sets of affinity group terminally functionalized layer
forming monomers in which different affinity groups correspond to

different intermediate (intervening) structural member composition,
and hence physical properties, layered and multilayered

Rapid Formation of Precise Thickness Multilayers:

5   Where multilayers comprising many molecular layers with monolayer
precision are required, methods more rapid than sequential monolayer
deposition are desirable. This may be accomplished by forming a
Langmuir-Blodget film on a solid surface which may later be dissolved
by a solution which will not disrupt or destabilize the overlying

10   layer. This surface is again used to lift an interfacial layer, such
that there are now two layers situated on said surface. Dissolving
such a substrate permits the overlying layer to be situated at a phase
boundary, and is favorably accomplished by slowly lowering said
substrate into said solution which dissolves it, such that once it is

15   dissolved, said overlying layer is situated at said phase boundary.
After the preceding steps, there would be two stacked monolayers at
this point. Conditions (e.g. temperature, composition, etc.,) are
adjusted such that a dissoluble solid may again be passed through said
solution without dissolution, to capture said overlying layer

20   previously situated at said phase boundary. Said surface with said
overlying layers is again used repeatedly to capture layers of a
precise thickness, which thickness is determined by the preceding
liberation step. The number of capture steps and the layer thickness
thus determine the precise number of monolayers added before a

25   substrate is dissolved, liberating the resulting multilayer at said
phase boundary. Thus thickness may increase multiplicatively rather
than in increments of a single monolayer or a single bilayer. Thus,
such a method comprises the steps of: forming an interfacial layer,
e.g. from amphiphilic molecules at an air water interface; depositing

30   such a layer on the flat surface, preferably of a dissoluble solid;
repeating this deposition step at least one more time; liberating the
multilayer thus produced from said surface, preferably such that it is
situated at a phase boundary and preferably by dissolving said solid
in a solution which will not disturb the structure of said multilayer;

35   and, repeating this process one or more times with said multilayer,
such that such repetition yields a multiplication in the thickness of
the preceding multilayer.

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The principal limitation to this method is that the sum of the area
of the monolayers of the resultant multilayer will be conserved with
respect to the area of the initially formed monolayers or bilayers.

Means other than ordinary dissolution may also be used to liberate
5   multilayers. For example, a substrate used with this embodiment may
be of a polymeric composition comprising photolabile linkages. Said
polymeric composition comprising photolabile linkages is preferably
chosen such that the photodegradation products will be soluble in the
solution used to form interfacial films or to support thus liberated
10   films, and to not bind with or otherwise interfere with these films.
Subject to these limitations, a wide range of compositions are
suitable for substrates. Where substrates are of polymeric
composition, these are most preferably cast with a highly flat
surface, simple examples of which include glass and freshly cleaved
15   mica. After each addition of layers as described above, substrates
are floated or otherwise held at the surface of the solution to
support the overlying film supported by said substrate after
dissolution of said substrate, and the substrate is exposed to a
sufficient intensity of light of a frequency chosen to cause the
20   degradation of said photolabile linkages for a sufficient length of
time to effect complete dissolution of said substrate, with further
time being allowed as necessary for degradation products to diffuse
away in this solution from the interfacial layer.

Note that such a process may alternatively avail a release layer,
25   which may be produced as a self-assembling monolayer, for example
comprising photolabile or thermolabile linkages situated on the
surface of an insoluble substrate. Here, said substrate and release
layer are lowered into a solution which will support the overlying
layer or film as a physical treatment disrupting said release layer is
30   effected such that said overlying layer or film is lifted by said
solution from said substrate, the bonds comprised in the structure of
said release layer having been disrupted. The composition of said
release layer is preferably chosen so that the disrupted layer
subsequently produced is well wetted by the solutions used, and such
35   that the free degradation products (those not bound to the substrate
surface) are well solvated by the solutions used.

Additional Embodiments:

Molecular and Supramolecular Synthesis with Positional Control:

This embodiment describes first devices for the parallel positional synthesis of molecular and supramolecular objects or articles (termed

5   workpieces or workpiece precursors) which may also be devices, methods for the fabrication of said first devices according to the general microfabrication methods of the present invention, and methods for the use of said first devices to effect positionally controlled molecular and supramolecular synthesis. Terminology used will include

10 terminology from the field of art of scanning probe microscopy.

Surfaces arrays of Z-actuators, each under independent control are produced, for example, by the foregoing methods of the present invention according to the following design. A capacitance based actuator is produced by forming electrodes on a surface which serves

15 as a first plate of a parallel plate capacitor. Two categories of capacitive actuators are possible: unfilled (i.e. air or vacuum gap) capacitors where the coulombic force between capacitor plates arising from the charge separation due to applied voltage difference is opposed by forces associated with mechanical flexure of a second

20 capacitor plate partially or completely suspended over said first plate such that a cantilever arrangement results; and filled capacitors wherein said first plate is coated with an elastiomeric or other compressible substance, according to the molding or casting methods of the present invention, upon which a second plate of a

25 parallel plate capacitor is formed, where compression of said elastiomeric or other compressible substance opposes the coulombic forces resulting from charge separation across the gap of said parallel plate capacitor. Note that for filled capacitors, composition of the substance between capacitor plates may further be

30 chosen according to dielectric properties in addition to mechanical properties. The functional characteristics of both categories of actuators will be highly dependent on geometrical factors and on materials properties, providing a large range of realizable device characteristics according to tradeoffs elected in design and

35 fabrication. Each such capacitive actuator may be placed under control of a different voltage signal, under electronic control, favorably with the electronic circuitry effecting this control integrated into the same array device, according to conventional

electronic device integration design practices and, for example, the
microfabrication methods of the present invention, such that each
capacitive actuator may be translated to a position independently of
other capacitive actuators in the same array. For purposes of the
5   present description, the direction normal or perpendicular to the
surface upon which said array of actuators is formed shall be denoted
as the Z-axis, which is also the axis substantially perpendicular to
the plane of the plates of said parallel plate capacitor actuators.
An integrated array of Z-actuators is thus produced on a substrate
10 surface.

The portions of said Z-actuators furthest from the substrate (i.e.
the topmost capacitor plate) may further comprise or be in
communication with a small cantilever, the purpose of which is to
facilitate the measurement of forces acting upon any tips associated
15 with said cantilever. Said forces may conveniently be measured by
reflecting a laser beam from the bottom surface (i.e. the surface
nearer to said substrate, which in this instance is chosen to be
transparent) and monitoring and changes in the reflected angle of said
laser beam (e.g. with a split photodiode arrangement as is commonly
20 used as detection means in AFMs), or by other means which have been
employed in the design of AFM cantilever deflection monitoring means
(e.g. capacitive detection, tunneling detection, piezoresistivity
change detection, etc.,.)

In the case of tunneling detection, a cantilever is coated with a
25 conductive layer on the bottom surface of said cantilever, which is
juxtaposed with a tunneling contact. The extent (proximity) of said
tunneling contact to said conductive layer on said cantilever may be
self-aligned by electroplating at said tunneling contact until a
desired current is observed between said conductive layer and said
30 tunneling contact, while said cantilever is at its equilibrium
(undeflected) position, optionally followed by a brief reversed
potential to dissolve a very small portion of the deposited metal from
the surface of said tunneling contact to establish a predetermined gap
size. Detection methods relying on such structures may additionally
35. rely on detection of variation in field emitted current between said
conductive layer and the conductive structure termed a tunneling
contact above in the case of tunneling current based deflection
detection.

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The signals corresponding to the deflections thus detected may, as
in conventional AFM systems, be used to adjust the position of said Z-
actuators to restore a desired set point value. As will be apparent
to those skilled in the arts of SPM and SPM design, other regimes of
5   AFM and SPM will be possible with the appropriate modifications or
extensions of the present array format.

The externally exposed surface of the topmost plate of said
parallel plate capacitor, or layers formed upon said externally
exposed surface may be further patterned such that a confined surface
10 region, of predetermined size and surface elemental or chemical
composition is produced in communication with the moving portion of
said capacitor actuator. In the simplest case, a masking pattern may
be formed on the exposed surface of a gold said topmost plate such
that only a small region, for example smaller than 100nm by 100nm
15 square of bare gold remains exposed. This surface thus comprises a
localized region or pad in and X-Y array of said actuators, which may
be translated in the Z direction independently of other similar
localized regions or pads of other actuator array elements.

20   Patterned Molecular Tip Arrays:

In one variant of this embodiment, said square of bare gold may be
used as a target for the random adsorption of organothiol molecules or
molecular complexes. Such adsorption is preferably carried out such
that deposition of said organothiol molecules or molecular complexes
25 occurs at a rate of one per pad or region, i.e. preferably exactly one
per actuator, but at least on average one per actuator in the case of
random deposition. In other variations, small objects may be
substituted for said organothiol molecules or molecular complexes, in
which case the said externally exposed surface or coatings thereupon
30 are chosen so as to stably bind the corresponding said small objects
selected. Said small objects may be selected from among:
macromolecules, enzymes and conjugates thereof, biological receptors,
immunoglobulins or fragments thereof or conjugates thereof, colloids,
nanocrystallites, polymeric beads, dendrimers, fullerene derivatives,
35 nanotube derivatives, mesoscopically structured single objects or
other small objects of predetermined geometries or geometrical
families. These molecules, molecular complexes or other small objects
serve as an extremities or projections in communication with actuator

pads; these will be generically referred to herein as tips. Note that
other types of tips include any of the above small objects in
communication via a flexible molecular linker to a chemical functional
group which binds stably to said pads, such that said small object is
5   positionally constrained according to the flexibility and tethering of
said linker, but not precisely positioned. Further, another type of
tip may be formed by electron beam deposition of vaporized substances
onto said pads.

Note that actuator arrays may homogeneously consist of a single pad
10   type decorated by a single type of tip, multiple pad types (i.e.
multiple pads of each type where each of said types has a different
surface chemical composition) decorated on a one-to-one composition
basis by corresponding multiple small object types, or may comprise
different pads decorated, according to foregoing spatially
15   predetermined copolymer synthesis methods, with distinct
oligonucleotides, polypeptides or other copolymers with distinct
binding properties.

Arrays of workpiece pads are produced by forming small exposed
20   regions of a surface of a convenient composition on a substrate
surface, such that said workpiece pads may line up with actuator pads
and/or tips of the above actuator arrays. Workpiece pads may also
comprise or overlie actuators, which may for example serve to tilt
said workpiece pads relative to said tips, or may merely comprise pad
25   arrays which facilitate the colocalization of tip and workpiece,
depending on the design of the article of fabrication and the tip or
tips used.

In particular, molecular tips within tip arrays (tip flats) such as
those described by Drexler[53] may be selected according to the tilt of
30   said workpiece pad effected by one or more actuators which adjust the
angle of the plane of said workpiece pad relative to the normal
defined by the Z-actuator of the tip pad. Note that the tip arrays
described by Drexler pertain to conventional atomic force microscopes
and cantilevers used therewith, said cantilevers modified to further
35   comprise said tip flats. To perform the corresponding synthetic
operations in parallel, rather than on a single workpiece, the present
invention provides that an array of actuators positions molecular tips
or arrays thereof along the Z-axis, while one or more actuators

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associated with each workpiece pad orients said workpiece pad to orient said workpiece pad such that one molecular tip of the opposed array is selected according to the orientation dependent proximity. Such molecular tip arrays may, following Drexler, be situated on a

5   curved surface, here each in communication with one of said Z-actuators, where the approach of a particular molecular tip in one of said arrays is selected according to the tilt of the workpiece relative to said one of said arrays which selects a distinct normal to said curved surface, thus selecting said molecular tip situated on

10 said curved surface at the locus of the line normal to both said curved surface and said workpiece pad (i.e. the point of contact as said curved surface would approach said workpiece pad, assuming convex shape.) Thus, provision of workpiece pads comprising one or more actuators effecting tilted orientation of said workpiece pads relative

15 to the Z-axis translation of said Z-actuators of said tip pad arrays permits the use of arrays of tip arrays in the parallel modification of arrays of workpieces. If such tilt is not effected by arrays of actuators, only one workpiece may be produced at a time benefiting from the selection of particular individual tips (one at a time) from

20 among the multiple tips in a tip array, because the appropriate workpiece substrate alignment for one workpiece will preclude interaction of other workpieces on the workpiece pad array from being juxtaposed with the pads of said tip pad array. The particular advantage of being able to select individual tips from an array of

25 tips in the modification of a workpiece accrues from the ability thereby to selectively interact said workpiece with a different, predetermined chemical functionality or composition of said individual tips, while maintaining a deterministic relationship between the spatial location of said tip array with said workpiece (i.e.

30 eliminating the need to locate a workpiece upon changeover of tips.) An array of tilt actuators permits such advantage to be availed in combination with the advantages of parallelism accruing from the simultaneous positionally controlled modification of workpieces situated upon said workpiece pad array comprising said array of tilt

35 actuators.

Local Vertical Positioning and Global Lateral Scanning of Probe Arrays:

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Where conventional scanning probe microscope designs, including microfabricated implementations thereof,[54,55] comprise tips each having X, Y and Z actuators, greater ease of fabrication and greater X-Y positioning accuracy may be achieved in array format by producing

5 an array of tips each situated on a distinct, independently controlled and monitored Z-actuator, where said array of tips overlying such an array of Z-actuators is translated by a single X-Y positioning means. While such an arrangement precludes independent X-Y motions of the individual tips of said array of tips, the elimination of separate X-Y

10 actuators for each tip of said array of tips permits a higher areal density of tips (or tip pads in the terminology adopted above, as distinct from the molecular tips of a molecular tip array, which would be situated on a single tip pad of said tip pad array) than would be possible where redundancy of X-Y actuators occurs. Retention of

15 independent Z-actuators in such a design permits maximally precise alignment of different tips with the corresponding opposed workpiece pads without variations in interaction forces between tips and workpieces which would result when cantilever flexion compensates for Z positional variation of said workpieces relative to said tips.

20 Where said X-Y positioning means is a piezoelectric tube scanner, additional care must be taken in calibration and scanning voltage waveform generation to ensure that deviations from planar orientation of the tube opening do not cause a rocking motion of the array mounted thereupon (i.e. compensation in the Z-axis at various points around

25 the height of the tube scanner as a function of X and Y position). Less conventional piezoelectric bimorph actuators or capacitor actuators are thus favored in this regard, though other measures may ensure proper operation with piezoelectric tube scanners.

30   **Self Alignment of Molecular Tip with Molecular Workpiece:**

Where molecular tips or other molecular positioning members are randomly situated on said tip pads of said tip pad arrays, alignment with the molecular precursors of workpieces or workpieces situated on

35 the workpiece pads of said workpiece pad array must be accomplished. This is most favorably accomplished by initially positioning said molecular precursors of workpieces on said workpiece pad array with said molecular tips or other molecular positioning members on said tip

pad arrays. Placement of said molecular precursors of workpieces or
said workpieces on said workpiece pads of said workpiece pad array by
said molecular tips or other molecular positioning members ensures
correct relative positioning of said workpiece precursors or

5   workpieces relative to said molecular tips or other molecular
positioning members and the reactants later carried thereupon. Thus,
random variations in the precise positions of said molecular tips or
other molecular positioning members on the pads of said tip pad arrays
pose no difficulty in alignment because such placement causes

10 corresponding variations in the precise positions of said workpiece
precursors or workpieces according to those of said molecular tips or
other molecular positioning members, i.e. positions of molecules or
complexes thereof on arrays are replicated, and such replication
measures ensure good positional match between molecular tips or

15 molecular positioning members and workpieces.

       As an example, a molecular workpiece precursor, comprising a thiol
group, may be deposited onto a workpiece pad comprising an exposed
gold surface by a molecular tip, such that the resulting position of
association of said thiol with said gold surface corresponds to the

20 position on said tip pad or cantilever of said molecular tip. In a
further extension of this example, said workpiece precursor may
additionally comprise a cleavable linkage susceptible to a particular
predetermined physical or chemical treatment, and a first reactive
group, for example a hydroxyl. A second molecular workpiece precursor

25 molecule, for example of identical composition, may be deposited by
the same process at a second location on said workpiece pad. Thus,
two predetermined reactive groups are placed in a predetermined
arrangement on said workpiece pad. As desired, a third molecular
precursor molecule may similarly be positioned at a third point, in

30 which case the three positioned reactive groups thus fix a coordinate
system admitting no gross rotations (i.e. further exclude the rocking
motions possible in structures anchored with only two reactive groups
fixed on a surface.) Following the placement of such workpiece
precursors, molecular components may be reacted with the reactive

35 groups of said workpiece precursors according to the methods described
below.

       Note that further self-alignment may be achieved at an earlier step
by replicating the flat surface of the substrate to be used to produce

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said workpiece pad array from that to be used to produced said tip pad
array. Such a step will ensure that local variations from planarity
(e.g. curvature) in the surface of said tip pad array are compensated
by the corresponding variations thus produced in the replicated

5   workpiece pad array substrate surface. The necessity of such steps in
practice will depend on the size of such surfaces and the ability to
obtain or fabricate ideally flat substrate surfaces as materials for
the production of tip pad arrays and workpiece pad arrays.

10   Constraint-Based Simplification of Positional
          Synthesis:

     A more immediately practical approach to the positional synthesis
proposed by Drexler fabricates structures from structures larger than
single atoms, and avails conventional solution and solid-phase

15   chemistry where positional control is not essential. These methods
permit the synthetic fabrication of extended, complex heterogeneous
structures which would be difficult or impossible to produce by known
art chemical methods.

     Most conveniently, the size of molecular components or reactants

20   and the linkers which hold these in communication with said molecular
tips or molecular positioning means are chosen to be of a sufficiently
great size that angstrom positioning accuracy is not strictly required
to exert positional control over synthesis. Instead, said linkers,
which may for example be composed of a polymethylene (polyethylene)

25   polymer chain or other preferably straight chain flexible polymer
chain, which are attached via some cleavable linkage to said molecular
components or reactants, constrains said molecular components or
reactions to a volume defined by the linear length of said linkers
(and the size of said molecular components or reactants) with a

30   probability distribution (or effective concentration as a function of
position within said volume) which may be predicted using Flory
theory. Said volume to which said molecular components or reactant
may be termed a constraint volume. Positional control over synthetic
reactions involving said molecular components or reactants and said

35   workpieces or workpiece precursors inheres in the X-Y translation of
the substrate comprising said tip pad array and the Z-translation of
said tip pads, such that the resultant three dimensional positional
control translates said constraint volume which contains said

molecular components or reactants (more specifically, the reactive
chemical functional groups thereupon) into proximity with the desired
reactive sites on said workpiece. In other words, control over
translation of said constraint volumes is used to select which

5 reactive sites of said workpieces are reacted. Thus, the resolution
of such a method is limited by the extent of said constraint volume,
and workpieces are preferably designed according to said resolution
(otherwise resultant workpieces will obey the corresponding
probability distribution related to the number of possible sites

10 within an approached constraint volume and the effective concentration
distribution at each of said possible sites.)

Note that such a linker may alternatively have a large central
portion of their length composed of a rigid polymeric member (with
terminal flexible polymeric linkages), in which case the attached

15 molecular components or reactants are confined rather to a shell,
which may be termed a constraint shell. The configuration of said
constraint shell is determined by the structure of the partially rigid
said linker, i.e. defining a volume beyond which the terminally
situated said molecular components or reactants may not extend and

20 also a closest point of approach to the point on said tip pad at which
said partially rigid said linker is attached beyond which said
molecular components or reactants may not approach. In this case, the
effective concentration of said molecular components or reactants
within such constraint shells are correspondingly higher as compared

25 with the above described constraint volume corresponding to a
completely flexible structure. Note, however, that greater
positioning accuracy may be required in these instances, but such
accuracy should be well within that practically achievable with
current instrumentation.

30 After the positionally constrained reactions occur according to the
above described steps, communication of said linkers and said
workpieces, which results from any bonds formed by said reactions, may
be tested. This may be accomplished by monitoring any forces exerted
by said communication as said tip pad array is withdrawn from said

35 workpiece pad array or, alternatively, as said Z-actuators of said tip
pads are translated away from said workpieces. Said forces may be
monitored conveniently if each of said molecular tip (or said linker)
is in communication with a cantilever, the position of which is

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tracked by any known art method such as optical beam deflection,
monitoring of a tunneling current between said cantilever (coated by a
metal) and a tunneling tip, changes in piezoresistivity, changes is
capacitance where an electrode situated on said cantilever forms one
5   plate of a capacitor, etc.,.

Two possible cases occur. First, communication of said tip with
said workpiece may be detectable as cantilever deflection upon
retraction of said tip from said workpiece, while said retraction does
not sever said linkage. Alternatively, said retraction may sever said
10   linkage. These two cases will have different requirements and
advantages.

In the first case, a linker is preferably of elastiomeric
composition such that tension is gradually applied to said workpiece
as deflection of said cantilever occurs during said withdrawal. In
15   this way, gross severing of linkages, which would be expected with
applied tensions of less than 10 nN for single bonded linkages, are
avoided. Otherwise care must be taken to ensure that applied tensions
remain under a tolerable maximum to prevent such severing. In such
cases, once a reaction between said molecular component and said
20   workpiece has occurred, the tip remains in communication with said
workpiece, until chemical or physical treatment severs the resulting
linkage. Linkages (i.e. linkage composition) is chosen according to
the desired methods of linker cleavage. For example, linkers may
comprise bonds susceptible to cleavage by particular chemical
25   reagents, may be heat labile or may be photocleavable, e.g. comprising
esters to compounds such as nitroveratryloxycarbonyl compounds, such
that said ester linkages are cleaved by exposure to appropriate
wavelength actinic radiation.

In the second case, such as the case for molecular components bound
30   to said molecular tip or molecular positioning means via affinity
interactions consisting of weak forces (e.g. hydrogen bonds, van der
Waals forces, solvation energies) withdrawal will lead to severing
with a predetermined applied force, which will be detected as the
corresponding cantilever deflection. In the case of oligonucleotide
35   based affinity interactions, the observations of G.U. Lee, L.A.
Chrisey and R.J. Colton[56] are indicative of the kind of affinity
interaction severing forces expected. This second case, with
oligonucleotide binding, is preferable in that well characterized

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binding interactions may be used to effect rapid, reversible binding
of molecular components to molecular tips or other molecular targets
(i.e. molecular positioning moieties) comprising the complementary
oligonucleotide sequence.

5   In either case, reactant molecular components are situated on said
molecular tips or tip pads by contacting said tip pad array with a
solution comprising the said reactant molecular components to be added
to said workpieces during the subsequent reaction step and permitted
to bind to the available sites on said tip pad array, i.e. to

10   molecular tips or to the affinity groups thereupon.

Once a molecular component has reacted with the specified site on a
workpiece or workpiece precursor, and has been severed from the
molecular tip or molecular positioning means, if any workpieces on
said array of workpiece pads did not evidence communication with said

15   cantilever (i.e. negative deflection upon tip withdrawal), and yield
is to be maximized at the expense of the repetition of steps, the
preceding steps from binding of molecular components to molecular tips
or molecular positioning means on said tip pad array through said
withdrawal step may be repeated with the same molecular component and

20   positioning. Sites which had previously been reacted will lack active
reactive sites, while those which did not successfully react will
experience a repeated opportunity to react with the desired species.
After an arbitrary number of failures, a workpiece may be deemed to be
mispositioned or otherwise refractive to correct assembly.

25

Constraint based simplification applies not only to constraint of
reactants (i.e. molecular components) to tips, but also applies to the
design of molecular components themselves and the design of the
workpieces which they are used to assemble. A molecular component

30   will generally be bound to a workpiece at more than one location, i.e.
will generally have greater than one point of connectivity with said
workpiece. This is most simply and rapidly accomplished with only one
positioning step per molecular component, with the formation of
subsequent linkages constrained by the geometries of said molecular

35   component and the structure, at that step, of the workpiece. In other
words, a positionally controlled reaction links such a reactant
molecular component to a first reactive site on a workpiece, and the
structure of said reactant molecular component and said workpiece are

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such that the reactive groups on said reactant molecular component and said workpiece will react together for only one relative topological orientation of said reactant molecular component and said workpiece. For rigid structures (i.e. those comprising rigid polymeric

5   structures) simple design rules may yield such a result, namely, that the pairwise distances of all similar reactive groups are different and that once a first bond is formed, under positional constraint imposed by linkage to said tip, no incorrect second bonds to reactive groups on said workpiece may form after release of said reactant

10   molecular component from said tip. In other words, no rotation of said reactant molecular component about the first bond formed under positional constraint by said tip will yield proximity of reactive groups situated on said reactant molecular component to correspondingly reactive groups of said workpiece except that rotation

15   which permits the desired reaction and hence the desired connectivity.

Note that flexible components may also be employed according to such constraint-based design methods, but here flexibility entails that the positional constraints on reactions of these components obtaining after a first bond or linkage is formed between such

20   flexible components and a workpiece will concern the maximum possible reach of other reactive groups on said flexible components. Thus, the selection of rigid or flexible components for different parts of a workpiece structure will be according to considerations concerning both the desired structure and the preferred molecular assemble

25   strategy. Note, however, that with both flexible and rigid components, and also with components comprising both flexible and rigid regions, component sets may be synthesized comprising different topologies such as different degrees of branching, different lengths of branch arms, different chemical functionalization of regions or

30   location of said branch arms, etc.,.

Aside from the above described positionally constrained reactions, workpieces may be treated with other reactants or chemical modifying reagents from bulk solution. For example, reactive groups on molecular components may all be primary hydroxyl groups. Once said

35   molecular components are incorporated into workpieces (and released from said tips), and said tip array withdrawn from said workpiece pad array, said hydroxyls may be modified to be reactive to other hydroxyls, e.g. by reaction with bivalent cross-linking agents

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(provided in excess) comprising two acid anhydride or two acid
chloride functionalities. The first such functional group of each
such cross-linking agent molecule will react with each hydroxyl on
said workpiece, while the second such functional groups of each such
5   cross-linking agent molecule, now a reactive group on said workpiece,
will only react with approaching hydroxyls of new molecular
components. Of course, this entails that no two hydroxyls available
on a workpiece may be closer together than the length spanned by the
reactive functionalities of such cross-linking groups. Alternatively,
10 molecular components may carry protected functional groups, which,
once said molecular components are reacted onto said workpieces and
released from said tips, are deprotected by contacting said workpiece
pad array, as in the case of activation with cross-linking agents, in
this case with deprotection reagents. In either case, function groups
15 which were held inert with respect to each other to prevent unwanted
reactions between molecular components as these are prepared and bound
to said tips, are activated once on said workpiece. In the case of
acid chloride or acid anhydride chemistries, inert solvent media must
be chosen. Further, control over the steps at which functional groups
20 on said workpieces are activated or deprotected provides another
method of control over synthesis, i.e. a method by which a subset of
functional groups are held inert until a desired assembly step,
whereupon they are deprotected or activated by treatment with
appropriate reagents. It will be obvious to those skilled in organic
25 synthesis that other reactive chemistries, as will as multiple,
concurrently used chemistries will be possible and often preferably
advantageous over the simple example presented here merely for
purposes of illustration.

      Note that molecular tips and any affinity groups thereupon may thus
30 be spared from exposure to highly reactive groups (e.g.
oligonucleotide moieties of molecular tips need never be exposed to
acid chloride containing compounds, which would inactivate said
oligonucleotides for proper binding).

      As an example, reactive groups may be primary hydroxyls on alkyl
35 chain linkers, rigid structural members may comprise polyparaphenylene
derivatives[57] or the phenylacetylene polymers disclosed by J. Zhang,
J.S. Moore et al.[58], in either case comprising components with
structures synthetically accessible, for example, by the methods of J.

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Zhang, J.S. Moore et al.,.[59]   Affinity members for reversibly binding
these components to molecular tips may comprise oligonucleotides,
oligopeptides, peptide nucleic acid oligomers or small molecule
haptens joined to said components (e.g. via methylene linkers),

5   according to the complementary affinity members on said molecular
tips. Other molecular component structures of interest in the
construction of molecular devices by such assembly methods include
rotaxanes comprising macrocycles which themselves comprise reactive
groups, and catenanes. Molecular components based on these

10   topological compounds provide structures useful as moving parts in
objects and devices fabricated by these synthesis methods.

     Affinity groups such as oligonucleotides may be included within the
structures of said molecular components or may be positioned by tips
into proximity with proximity selected reactive groups on said

15   workpieces which will react with linkers (e.g. at terminal hydroxyls)
attached to said affinity groups. Note that if workpieces are to
subsequently be exposed to reactive compounds, said affinity groups
may be provided with any potentially reactive functional groups
protected by protecting groups, which may be removed in a final step

20   after such exposure. Note that such affinity groups to be included in
the final structure of said workpiece must thus be protected to ensure
that these are not derivatized in undesired ways by synthesis
reagents, but affinity groups used to bind reactant molecular
components to said molecular tips or molecular positioning means may

25   be sacrificed. Said affinity groups used to bind reactant molecular
components to said molecular tips or molecular positioning means may
be in communication with the remainder of the structure of the
reactant molecular component which they serve to target to said
molecular tips or molecular positioning means via linkers cleavable by

30   predetermined physical and/or chemical treatments (e.g. hydrolysis of
an ester linkage) such that these moieties may be removed from said
workpiece after they are no longer needed. Of course, such removal
must be accomplished according to chemistries which do not adversely
affect the remainder of the workpiece structure, and are thus to be

35   chosen according to such design considerations.

     Note also that similar positional constraint methods may be used to
effect positionally controlled deprotection of reactive groups, such

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that the position of a tip is used to select which reactive group or
groups on a workpiece, out of several groups of similar composition at
different locations on said workpiece, will be active during a
subsequent reaction step where the reactant molecular components react
5   with such deprotected or activated chemical functional groups on said
workpieces directly from solution. This variation adheres to the same
basic principle of selective positional modification of chemical
functional groups, here preparing their activity for subsequent
synthetic steps. For example, chemical functional groups may be
10 primary amines, which may be protected by esterification. Here, a
molecular tip may comprise or may be linked (e.g. by known art
conjugate chemistry) to a protease enzyme molecule, such as Proteinase
K. Only those primary amines within the reach of such enzyme
molecules tethered to a molecular tip or tip pad are susceptible to
15 enzymatic deprotection, and hence only the subset of such amines on
said workpieces will be deprotected. Other catalysts such as
ribozymes, metal tip surfaces, or metallic colloids partially embedded
in polymer matrices may similarly be used, as may tethered molecules
which effect deprotection by non-catalytic reactions.
20

Nanoscale Data Pattern Replication and Readout Methods and Means:

The large data capacities realizable with the various data storage
25 methods comprising the use of scanning probe technology based data
readout are not accompanied by a commensurate increase in the rate of
data recording except where large numbers of scanned probes are used
to record a data pattern onto a storage medium surface. Because one
particular advantage of such storage technologies is in applications
30 related to data publishing, a rapid method for the duplication of
predefined bit patters readable with such readout technologies would
be desirable, as has been the case with read-only optical data storage
technologies.

The methods of the present invention are well suited to the
35 fabrication of surfaces with inscribed nanoscale data patterns
readable with various scanning probe readout means. These include
near-field photon transfer (corresponding to NFSOM), electron
tunneling and electron field emission (corresponding to STM and field

emission mode STM), and surface profile detection (corresponding to AFM and variations thereon).

In the case of photon transfer, a transparent surface such as glass or polycarbonate is coated with a metal film, which is patterned by the foregoing methods of the present invention for the spatially predetermined protection or masking of metallic film surfaces from etchants such that the desired bit pattern is formed comprising apertures in said metallic film and intact regions in said metallic film which reduce or eliminate the local transmission of light. Such a bit pattern may be read out by conventional NFSOM apparatus or variations thereupon, or the near-field detection means described below. The patterned said metal layer may mask the local transmission of photons to a probe aperture which conducts received photons to a detector, said photons originating from a source on the opposite side of the transparent material comprising said transparent surface, in which case said metal layer serves as a photon transmission mask. Alternatively, said pattern in said metal layer may mask the transmission of photons emitted due to the fluorescence of compounds in said transparent material excited by photons either emanating from an NFSOM tip aperture which may be the same aperture used for the detection of fluorescently emitted photons or emanating from a source on the other side of said transparent surface.

Similarly, in the case of electron tunneling transfer or electron field emission transfer, a metallization pattern may similarly be formed, here on an insulator, or alternatively a relief pattern may be formed in one or more metal layers with all exposed surfaces having conductive properties. In either case, a bit pattern which may be replicated by the methods of the present invention is encoded in such a surface relief pattern. Such a bit pattern may be read out with instruments such as STMs, microfabricated STMs or the array devices disclosed herein comprising plural Z-actuators each associated with a tip, which entire array is scanned across a juxtaposed surface by an X-Y positioning means and wherein data patterns are detected by monitoring, with electronic means, voltage, current or height of each tip either singly or in the various possible combinations and where said current may be either a tunneling current or a field emission current according to particular implementations.

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In the case of AFM or other surface height responsive data readout means, relief surfaces comprising surface patterns are replicated by casting, injection molding or embossing methods.

In all of these cases, an original relief comprising the desired
5   bit patterns may be formed by scanning probe technology based patterning methods such as those of related art reviewed above.

By such methods, large numbers of replicas of an initial surface pattern encoding information may be rapidly and inexpensively produced, permitting economical data publication in high density
10   formats readable by scanning probe based data readout technologies.

Simplified Method and Means for Data Readout by Near-Field Optical Scanning:

A CCD array, fabricated by methods of the present invention or by
15   conventional methods may be modified to serve as a highly parallel detection means for optical data readout relying on near-field photon transmission through a small aperture. An array of waveguide elements, for example composed of PMMA, is molded onto the surface of said CCD array such that each photodiode of said CCD array will
20   capture photons conducted by each of said waveguide elements. The array comprising said waveguide elements must therefore be aligned with the photodiode array of said CCD. Gaps between said waveguides are filled in with, for example, an opaque polymeric material, or may be electroplated provided this will not cause short circuits in said
25   CCD, which may be designed to prevent such problems. Said waveguide element array and any material filling said gaps are formed such that a substantially flat surface results. A metal film, preferably of gold, of sufficient thickness to prevent appreciable transmission of photons is then deposited onto said surface. Said film is then masked
30   such that only one small pinhole will be etched overlying each photodiode and associated waveguide; said pinhole will serve as an aperture limiting photon tunneling. This may be accomplished by patterning with organothiol compounds using an elastiomer, according to the method of Whitesides and co-workers, followed by an acid etch
35   step, by the related multilayer vetoing methods described herein, or by the other patterning methods disclosed herein. Thus, an array of pinholes of predetermined size and location is fabricated with associated waveguides, which conduct any photons received at the

aperture formed by said pinholes to the photodiodes of said CCD in a
one-to-one manner.

Such an array of pinholes with associated waveguides may also be
fabricated by LIGA methods and variations thereupon, and then situated
5   in an appropriately aligned manner onto a CCD array surface.
Alternatively, the same type of structure comprising an array of
pinholes with associated waveguides may be fabricated by other molding
and electroforming methods described herein and then, similarly,
situated in an aligned manner onto a CCD array. Such an array may be
10   termed an aperture limited CCD array (ALCCD).

The CCD array devices used in this aspect of the present invention
are preferably of high sensitivity, such that a reduced number of
photons must be received by each photodiode to register a signal.

Such an ALCCD may be used for data readout by juxtaposing the
15   surface of said ALCCD comprising said pinhole array to a patterned
metal film surface of a transparent material such glass, such as that
described above which pattern may be replicated as described above.
Said pattern metal film surface is held parallel to the surface of
said ALCCD comprising said pinhole array, at a distance of less than
20   one half the wavelength of the light employed and preferably at a
distance of less than one-quarter of this wavelength. Smaller
separations are further preferred. The region of said patterned metal
film opposite each pinhole on the surface of said ALCCD is either be
coated with said metal film or may expose the underlying said
25   transparent material, which distinction provides for the detection of
the corresponding data pattern. Thus, the density achievable with
this data readout apparatus and method is primarily determined by the
dimensions of said pinhole, which limits the photons thus received to
those originating from narrow exposed regions.

30   Said patterned metal film surface is scanned, in a manner
maintaining parallel alignment with the pinhole array surface of said
ALCCD, such that an area corresponding to the size of each CCD array
element passes opposite each pinhole, by repeatable positioning means
such as piezoelectric or capacitor actuators. In other words, said
35   patterned metal film surface is scanned in the X and Y while
maintaining a substantially constant Z separation, such that the
signal received by each photodiode of each CCD element correlates,
according to scan timing, with a particular position on said patterned

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metal film surface. Scanning is conducted slowly, such that said CCD
captures the bit image corresponding to a particular position of said
patterned metal film surface, which is then read out from said CCD
according to conventional methods associated with the use of such
5   electronic imaging or memory devices. Thus data rates will primarily
depend on the device characteristics of the CCD used. The X-Y
location of said patterned metal film surface relative to said ALCCD
are then translated by incremental scanning motion of said actuator,
and the bit pattern associated with this subsequent position is then
10 similarly collected.

In the simplest instance of this method, photons originate from a
source on the opposite side of said patterned metal film than said
ALCCD, with transmission occurring through exposed regions of said
transparent surface as it is masked by said patterned metal film. The
15 number of photons received by each CCD element are limited by the
relative size of said pinhole to the area of each CCD element. Thus
it is desirable that either high intensity sources are used to
compensate for this limitation, or that incident light be focused so
as to reach the pinholes situated on the surface of said ALCCD at a
20 sufficient areal intensity. This may be accomplished by the use of a
microlens array, for example such as that described by H.A. Biebuyck
and G.M. Whitesides,[60] with appropriate focal length of the lens
elements which it comprises, such that incident light is concentrated
as it passes through said patterned metal film surface and reaches
25 said pinholes on said ALCCD surface. Said microlens array is thus
constructed at a similar density as the CCD which is used, and is
aligned with the surface of said ALCCD such that said pinholes are
each at the focal point of the corresponding lens. In this case, said
patterned metal film surface is situated between said microlens array
30 and said ALCCD, and remains in a fixed position relative to said ALCCD
as said patterned metal film surface is scanned between said microlens
array and said ALCCD surface.

Method and Means for the Ordered Dissection of
35 **Chromosomes, Chromatin and Polynucleotides:**

A relief is formed by replication methods at the surface of an
elastiomer polymer composition, comprising a pattern of parallel lines
comprising narrow raised regions generally of less than 200nm in width

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and wider sunken regions preferably of greater than 1 micron in width.
The aspect ratio of said narrow raised regions may be reduced by
sloping the surface of said wider sunken regions in proximity to said
narrow raised regions. Said polymer composition is chosen such that
5   the resulting molecular network comprises chemical functional groups
which may be reacted with cross-linking reagents to attach molecules
to the surface of said relief. An appropriate cross-linking agent
comprising a central linker of predetermined length, preferably
shorter than 50nm is coupled to a nuclease enzyme (such as DNase I or
10 a type II restriction endonuclease, preferably recognizing a 4 base
restriction site) according to known art conjugate chemistry. Said
cross-linking agent is chosen so as to link said nuclease to said
elastiomer polymer comprising said chemical functional groups. A
small volume of a solution of said cross-linking agent conjugated to
15 said nuclease is spread on a surface as a thin layer in the pattern of
a single stripe, preferably of less than a few millimeters in width.
Said relief is contacted with said stripe of said conjugate on said
surface, oriented with said parallel lines perpendicular to said
stripe, such that nuclease molecules are linked to the surfaces of
20 said narrow raised regions along the region juxtaposed with said
stripe. The solution used to form said strip may favorably contain a
dye or pigment to facilitate identification of the enzyme modified
region, or other marking methods may be used to accomplish such
identification. Thus, a relief comprising parallel lines and trenches
25 of predefined size and location is produced with a predefined region
of raised features upon which nuclease molecules are situated.

Linear or linearized DNA molecules or chromatin are treated so as
to be immobilized at specific regions along their length, preferably
near to one distinct terminus, and optionally derivatized with beads
30 at the opposite terminus. For example, these may be hybridized with
an oligonucleotide comprising a first affinity moiety such as biotin
moiety and one or more psoralen groups (where plural groups are
preferred to favor the formation of covalent linkages with both
strands of the DNA double helix). After hybridization with said
35 oligonucleotide, said psoralen groups are caused to react with said
DNA to form a covalent linkages. Streptavidin is bound to a glass
surface in a narrow stripe (<1 micron in width) shorter in length than
the width of said strip of said conjugate above. Said DNA terminally

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linked to said biotin is then contacted with said narrow stripe of
streptavidin and affinity binding is permitted to occur. Then, for
example, the immobilized DNA sample is optionally then briefly
subjected to degradation by a 5' to 3' exonuclease, followed by

5   treatment with a polymerase and second affinity group modified
nucleotide triphosphates to terminally decorate said linearized DNA
molecules with said second affinity group. Where modification with
said second affinity group is performed, said immobilized DNA sample
is.then contacted with a solution comprising microscopic or sub-micron

10   diameter beads, which may be fluorescently labeled, surface modified
with receptors (e.g. immunoglobulins, etc.) according to known art
conjugate chemistry. Alignment of the immobilized DNA molecules is
then performed by known art techniques.[61] Said sample is then
subjected to mild fluid flow in the direction perpendicular to said

15   narrow stripe of streptavidin, as said glass surface is withdrawn from
the liquid (or the liquid otherwise passes away from said DNA sample
with an interface traveling in a direction perpendicular to said
narrow strip of streptavidin). The molecules of said DNA sample are
thus aligned in the direction perpendicular to said narrow stripe of

20   streptavidin, fully extended, and situated on said glass surface. The
same method may be applied to chromatin (first order packing of DNA
with histone nucleosomes) or with condensed metaphase chromosomes.
Straightening of terminally immobilized molecules may alternatively be
accomplished, where said molecules underwent the optional addition of

25   terminal beads as above, by methods such as those of T.T. Perkins et
al.[62] whereby beads situated on DNA molecules are manipulated using an
optical trap (also known as laser tweezers), or, where said beads are
of paramagnetic composition, by the application of an appropriately
directed magnetic field of sufficient strength to straighten, but not

30   so large as to disrupt, the sample molecular and supramolecular
structure.

Once sample molecules are end-immobilized to a precise,
predetermined region, oriented and extended by means such as those
above, the relief prepared above with raised narrow lines modified

35   with nuclease molecules is then contacted with said glass surface on
which said sample molecules have been straightened and aligned, in an
orientation such that the parallel lines of said relief are
perpendicular to said sample molecules, where said contacting is

performed under slight pressure to ensure sealing of the channels thus
temporarily formed. A gentle fluid flow is immediately established in
said channels, such that once a molecular region is freed at both ends
by cleavage by said nuclease molecules, it is carried down said

5   channel away from said nuclease molecules, which might otherwise
further degrade it. Said flow then carries the cleaved molecule
fragments to collection volumes formed in said relief structures at
the ends of said channels, which are arranged such that the location
of the channel in which a fragment was cleaved may be determined or

10   inferred from the location of said collection volume. Thus, the
collection volumes forms a linearly ordered set, which set comprises
an ordered arrangement of fragments of a well defined physical length
and precise ordering with respect to their original location in the
in-tact sample molecules.

15   Such a method may be repeated, as desired, with a slightly
different offset of said relief with respect to said narrow stripe of
streptavidin, such that different regions are occluded from the
collected sample set, to the extent that retention under said narrow
raised lines poses any difficulties.

20   Note that in all steps, care is taken to avoid shearing sample
molecules. Note further that slight differences in the positioning of
the ends of immobilized sample molecules entails that the ordering of
said fragments in a collection volume is better described as by a
positional distribution function with respect to the sequence of the

25   original in-tact molecules from which said fragments derive.
Nonetheless, this simple process yields ordered fragment populations
useful for establishing physical maps of genetic material and
producing ordered samples therefrom for purposes such as library
cloning or subcloning or other analysis techniques, and particularly

30   useful in genome sequencing projects.
     Two alternative genetic material dissection methods availing
micropatterned reliefs are also possible.
     In the first alternative, a relief comprising parallel raised
narrow lines and trenches, such as that described above, is formed.

35   This relief is then contacted with a first surface on which are coated
particles such as metallic or glass colloids or hard
microcrystallites, which are coated with chemical species capable of
crosslinking said particles to said relief. Thus, said particles are

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bound to the raised narrow regions of said relief. Genetic material
is immobilized on a second surface and aligned as above, and the
relief of the present alternative is oriented as above with said
parallel lines perpendicular to the direction of alignment of said
5   genetic material on said second surface and contacted with said second
surface under an applied normal force. Liquid is caused to flow
through the channels formed by said trenches and said second surface
as a result of the preceding contacting step. The relief is then
translated in the direction of said parallel lines as said normal
10   force is maintained and said liquid flow is maintained. Thus, the
genetic material is abraded and therefore cut by said particles, and
once cut, fragments are freed from communication with said second
surface and said relief, such that said fragments are carried by said
liquid flow down said channels to collection volumes. The cutting
15   action availed in the present alternative of this embodiment is in
direct analogy to the cutting of DNA molecules by an atomic force
microscope tip. Said second surface may be hard, such as glass, may
be elastiomeric; said second surface may comprise bonded particles
similar or identical to those used on said raised regions of said
20   relief, in which case the cutting action may be compared to a
scissoring action.

In a second alternative, a relief composed of opaque materials,
comprising parallel lines of narrow raised regions and trenches is
used, as above, to define flow channels when contacted under normal
25   force with a second surface, here of a sheet-like or flat material.
In this alternative, said second surface is transparent to radiation
of the appropriate frequency range but is coated (preferably on said
second surface, or instead on the surface not contacted with said
relief) with materials which mask said radiation. For example, said
30   flat material may be quartz coated with metallic mask materials and
said radiation may be X-radiation, or said flat material may be glass
coated with any material opaque to visible or ultraviolet light, which
is used for irradiation. In the case of X-irradiation, genetic
material exposed will be cleave directly due to double stranded
35   breaks. In the case of ultraviolet or visible light irradiation, said
genetic material is first bound by photochemical reagents which will
cleave DNA when activated by radiation exposure; where said masking
material is on said surface proximal to said relief, radiation may be

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transmitted to said reagents in the near field regime, permitting
resolution smaller than the wavelength used and equal to the masking
material feature resolution. The masking pattern used is designed
such that when said relief is contacted to said second surface, the

5   lines of narrow raised regions are juxtaposed directly to unmasked
regions of said second surface. Thus, said irradiation causes breaks
of said genetic material at the loci of said narrow raised regions,
which are in contact with said second surface, such that the fragments
thus produced will be freed within the channels formed by said second

10   surface and said trenches and carried away, by a fluid flow applied in
said channels, to a collection volume corresponding to the respective
channel.

Replicon Chimera Library Contiguity Analysis:

15   Articles produced by the methods of the present invention may be
used to facilitate the analysis of polynucleotide libraries such as
plasmid libraries, bacteriophage libraries, cosmid libraries, yeast
autonomous chromosome (YAC) based libraries and the like. In the
field of gene discovery and genome mapping it is often convenient to

20·   work with samples, generally in DNA form, which are fragmented and
incorporated into replicon molecules capable of being maintained,
replicated, expressed in and purified from appropriate host organisms.
The fragmentation of genetic material which occurs in the preparation
of such libraries, however, introduces difficulty into establishing

25   the order with which the fragments thus obtained occur within the
original, unfragmented polynucleotide sample. Further, mapping of
genetic material over millions of base-pairs yields information useful
in the field of genetics independent of the use of polynucleotide
libraries. Therefore, a method which both provides rapid and

30   economical genome or chromosome mapping, and is capable of determining
the ordering relationship between the replicon chimera comprising a
library would be of great use. Current art methods for accomplishing
such tasks generally rely on the probing of arrays or replicas of
clonal replicon-chimera isolates, whether in the form of colony or

35   plaque isolates or arrays of spots of genetic material from such a
library, generally rely on the transcription of RNA from bacteriophage
promoters at the borders of the replicon used to create the library
and directed such that sample fragments are thus transcribed.

Generally, such transcripts are transcribed with radiolabeled
ribonucleotides, and the RNA thus obtained is used to probe said
arrays or replicas; those array elements or colonies or plaques which
hybridize the radiolabeled RNA transcripts thus share common sequences

5   with the fragments from which said transcripts were obtained. Thus,
these methods determine which clonal isolates contain common
sequences, and by repetition identify series of clonal isolates which
are of contiguous origin. Because such libraries may contain well
over 10^4 distinct chimera, such information is rarely if ever

10   exhaustively determined for any given library, and is usually only
applied to small fractions of such libraries.

Copolymer arrays such as those disclosed in related art or produced
by the methods of the present invention may gainfully be applied to
the task of determining the linkage relationship between the

15   individual chimera of a library and also to chromosomal mapping
thereby. In this method, each replicon must be rendered uniquely
distinguishable, for example by the incorporation of a sufficiently
long random nucleotide sequence (e.g. 9 variable base pairs [which
need not all be contiguous] in a replicon designed for use in

20   preparing libraries of which $<10^5$ clones will be used.) Such a
sequence will be referred to as a tag sequence, but it will be
understood by those skilled in the relevant arts that different
tagging and discrimination methods (e.g. the use of epitope library
fused terminal-protein replicated viral or bacteriophage [e.g. PRD1,

25   phi29, adenovirus], probed with peptide or antibody arrays) are
possible, although nucleotide sequence based tagging with oligo array
discrimination is favorably convenient, and will therefore be
emphasized here. For nucleotide sequence based tagging, the tag
sequence is favorably placed such that it is transcribed under the

30   influence of a promoter in the replicon sequence and the appropriate
purified RNA polymerase.

A one dimensional array comprising all sequences complementary to
all possible tag sequences (or receptors capable of reversibly binding
all tag moieties) is formed by the above spatially controlled

35   copolymer array forming methods. Additionally, parallel to the lines
of said one dimensional array of said tag sequences, a plurality of
lines of oligos of different sequence are formed, preferably during
the same patterned synthesis steps. The sequences of the oligos in

each line are chosen to be useful in classifying the fragment
incorporated in a given chimera, or the transcript therefrom. The
hybridization of each classifying oligo with a sample molecule may be
regarded as a positive result from a classification test; each

5   fragment is tested by the full set of classifying oligos (i.e. all
lines), such that for n such lines such an array will be capable of
discriminating up to 2^n different classification results. Classifying
sequences are therefore optimally chosen to have about a 50% chance of
probing any given sample fragment or transcript, however large numbers

10   of classifying sequences (lines) may obviate this effort. The essence
of this method consists in uniquely identifying a chimera (according
to tag identity) and uniquely classifying one terminus of the
incorporated sample fragment, which classification information is
associated with the identity of the chimera to which it pertains,

15   where the use of copolymer arrays permits the concurrent analysis of
large numbers of chimeras or replicons. Correspondence between tag
identity and the regions of said lines of said classifying oligos is
enforced at the appropriate step with a relief (preferably of
elastiomeric composition) of parallel raised lines and trenches, of

20   sufficient spacing to accommodate both the tag probing oligo array
elements and the length of the polynucleotide fragments, juxtaposed to
the detection oligo array comprising said one dimensional array
comprising all sequences complementary to all possible tag sequences
and said lines of said classifying oligos, oriented such that said

25   parallel raised lines and trenches are perpendicular to said lines of
said classifying oligos.

A library of said chimera, in mixture form, is either transcribed
or fragmented, such that for each chimera, a first fragment type
comprising a tag sequence and a sufficient length of sample fragment

30   derived sequence is obtained. Such fragments are either produced in
labeled form (affinity, fluorescent, radioactive or other label) or
otherwise labeled before application to said array. Said lines of
said classifying oligos are covered to prevent contact with said first
fragment type as a solution containing said first fragment type is

35   contacted with a region of said array comprising said one dimensional
array comprising all sequences complementary to all possible tag
sequences, to which the molecules of said first fragment type are
permitted to hybridize under sufficiently stringent conditions to

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ensure high specificity and accuracy of tag identification. Unbound
sample molecules are washed away. Said relief is then juxtaposed to
said array in said perpendicular orientation. The hybrids formed
comprising said sequences complementary to all possible tag sequences

5   are then denatured and a flow of buffer solution is applied along the
channels formed by the trenches of said relief and the surface of the
substrate of said two dimensional array, and conditions permitting
stringent hybridization are then quickly re-established. Thus, the
molecules of said first fragment type, which have been confined to

10   particular channels according to the tag sequence which they comprise
are swept by said flow across said lines of said classifying oligos
and which they thus have the opportunity to hybridize with if and only
if they comprise sequences complementary to the sequences of any of
said classifying oligos. Unbound molecules are then washed away.

15   Hybridization of sample molecules to said array is then detected by
means which correspond to the labeling method used, and the
corresponding data favorably recorded by digital computer.

These data are then used to establish the linkage relationship
between chimeras according to similarity of classification: two

20   chimeras possibly overlap if the classification of said first fragment
type derived from each of them are consistent, i.e. share a
sufficiently large number of classifications not shared with an
improbably high proportion of chimera. In other words, two sample
fragments which partially overlap will be classified similarly

25   according to the hybridization of classifying oligos to the sequences
which they share. Combination of data derived from ensembles of such
overlap information permit the reconstruction of maps of larger
contiguous regions (such as chromosomal material) from which sample
fragments derive, and simultaneously yield information about which

30   chimeras (as identified by tag sequences) are derived from particular
regions of said maps.

As will be apparent to those skilled in molecular biology and the
art of recombinant DNA technology, many different tagging schemes are
possible. If care is not taken to ensure that tag sequences differ

35   sufficiently from sample fragment sequence, ambiguous results will be
obtained, but the ambiguity of such results will generally be
apparent, such that these results may be identified and ignored (e.g.
where an improbably high number of classifying oligos are hybridized

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within a given channel.) Such ambiguities may be reduced by
specifying tag and tag probing sequences by methods similar to those
applied to the design of sequencing and PCR primers, i.e. methods
which concern the selection of rare sequences and methods which
5   concern the production of degenerate probes (complex mixtures of
oligos conforming to some consensus sequence but each hybridizing to
different targets having the same complementary consensus sequence)
for rare sequences.

Molecules of said first fragment type preferably consist of
10 material from one extremity of the sample fragment incorporated into
the respective chimera. This minimizes the degree of overlap between
two chimeras classified as contiguous and thus minimizes the number of
chimeras necessary to represent the original linear sample, reducing
the work necessary for analysis. Note, however, that more redundant
15 information may be obtained by permitting a greater degree of overlap,
for example, by producing and then analyzing longer molecules of said
first fragment type. Thus, two extreme cases are possible: said
first fragment type comprising most all of a chimera; and said first
fragment type consisting of a minimal length of sequence to permit
20 useful classification. In practice, tradeoffs exist between these
extremes, and intermediate cases are probably preferable according to
the specific library and sample.

As will be apparent, each of said molecules of said first fragment
type must comprise said tag sequence and sequence from said original
25 sample from which said library was derived. Where said molecules of
said first fragment type are limited to one terminus of the sample
fragment sequence (i.e. one sample fragment sequence region adjacent
to one arm of the replicon vector used to construct the library), the
method of preparation of said first fragment type must ensure that the
30 tag sequence associated with one terminus may be correlated with the
tag sequence associated with the other terminus. At least two
instances conforming to this requirement are possible.

In a first instance, said molecules of said first fragment type may
be prepared by restricting aliquots of the library of said chimera
35 differently. Here, a the vector from which said library was prepared
comprises a first unique restriction enzyme site, which may be chosen
according to infrequent occurrence in the subject genome or original
sample, on one side of a tag sequence, and a second unique restriction

enzyme site, which may be chosen according to infrequent occurrence in
the subject genome or original sample, on the other side of a tag
sequence. In this case, a first aliquot is subjected to restriction
digestion with the enzyme specific for said first unique restriction
5   enzyme site, and a second aliquot is subjected to restriction
digestion with the enzyme specific for said second unique enzyme site.
Additionally, fragments may be shortened by any convenient method such
as shearing, random nuclease treatment, etc., to restrict the length
of polynucleotide fragments captured by tag probe oligos and
10 subsequently analyzed to convenient lengths. Each aliquot is analyzed
separately according to this aspect of the present invention, and
results are used to correlate similarly classed fragment sequences
found in said first aliquot with those found in said second aliquot.

In a second instance, each molecule of said vector from which said
15 library was prepared comprises two different, unique tag sequences,
each located near a terminus of said molecule, i.e. near the locus to
which a sample fragment is ligated. Thus, each tag sequence
corresponds to a terminus of a sample fragment in the library
constructed with said vector. Molecules of said first fragment type
20 having exactly one of said two different, unique tag sequences are
produced by known art methods which will be related in consideration
to the design of said vector (e.g. restriction digestion,
transcription from an artificial promoter, etc., according to
corresponding vector features in relation to said two different,
25 unique tags and the inserted said sample fragment.) Said molecules of
said first fragment type having exactly one of said two different,
unique tag sequences are analyzed according to the above library
analysis methods, probing in each case with the members of only one
tag probe set. Because each terminus is associated with a different
30 tag in each clone, the tag from one terminus must be correlated with
the tag of the other terminus. This second correlation step is
accomplished by the same method, binding chimera or fragments
comprising the vector termini with a one dimensional tag probing oligo
array complementary to the first tag set, where lines of different
35 classifying oligos in the array are replaced with lines of different
tag probing oligos, corresponding to those which bind tag sequences of
the second tag set.

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The foregoing embodiments and examples have been presented for
illustration rather than limitation, with the many possible
variations, changes and alternative embodiments which will be obvious
5   to those of ordinary skill in the relevant arts included within the
scope and spirit of the present invention. Thus, the scope and the
breadth of the present invention is intended to be defined by the
appended claims rather than the foregoing description.

10

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whereforth, I claim:

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Claims:

1.   A process for the fabrication of microscale or nanoscale
devices comprising the use of replicated patterned relief surfaces and
a mask forming step selected from the group consisting of: multilayer
vetoing of self-assembling monolayer patterns on metallic films;
polymerization of resist from patterned initiator compound regions;
casting of polymeric resist patterns on surfaces; injection molding of
resist precursors; embossing of a preformed resist film followed by a
mild etch to eliminate reduced thickness regions; curing of prepolymer
solutions wetting underlying patterns.

2.   A process according to claim 1 further comprising the
incorporation of alignment patterns into said replicated patterned
relief surfaces and the surfaces to which these are juxtaposed, where
said alignment patterns are selected from the group consisting of:
patterns which cause the appearance of Moiré patterns when overlapped;
interlocking surface relief patterns.

3.   A process for the patterning of three or more distinct
affinity species or reactants serving as precursors thereof on a
surface comprising one or more steps of contacting said surface with
one or more micropatterned relief surfaces.

4. A method according to claim 3 for the spatially controlled
synthesis of copolymer sequences on solid surfaces comprising the use
of relief surfaces to effect or prevent the physical contact of
reactants or reagents with regions of said solid surfaces.

5.   A method for the formation of molecular layers having
spatially predetermined compositional pattern, comprising one or more
steps of associating terminal affinity group functionalized molecules
with complementary affinity groups patterned onto surfaces or other
such molecular layers.

6.   A method for the replication of compositional patterns of
molecular layers produced according to claim 5.

7. A method for the positionally controlled synthesis of molecules or molecular complexes according to positional constraint design rules and comprising the use of positioning means which directly or indirectly bind reactant molecules, reactant precursor molecules, catalysts, enzymes, abzymes, ribozymes, deprotection reagent molecules, activator reagent molecules or protecting reagents, and position these relative to reactive groups situated on a workpiece or workpiece precursor comprising one or more molecules.

8. A method according to claim 7 where said positioning means comprises a microfabricated array.

9. A method for the spatially ordered dissection of genetic material comprising the immobilization of said genetic material to a predefined region of a surface.

10. A method according to claim 9 comprising the use of micropatterned surfaces.

11. A method for the determination of the ordering of clones or polynucleotide maps from a polynucleotide library comprising the use of one or more oligonucleotide or polynucleotide arrays.

12. A method for the fabrication and replication of data patterns on surfaces for read out by data readout means comprising scanned probes comprising the use of one or more patterned relief surfaces.

13. Method and means for readout of data patterns and surfaces prepared according to claim 12 comprising a charge coupled device and actuation means for translating said surfaces relative to said charge coupled device.

14. A method according to claim 11 further comprising the use of an elastiomeric relief comprising raised parallel stripes with microscale features.

15. A process for the patterning of two or more distinct materials or precursors thereof on a porous surface comprising one or

more steps of contacting said surface with one or more micropatterned
relief surfaces each of which is coated with a suspension of one of
said distinct materials.

16. A method according to claim 15 further comprising an
annealing step.

17. A method for the fabrication comprising the steps of:

    (a) creating one or more elastiomeric microreliefs;

    (b) coating said elastiomeric microrelief with a material
        from which an article is to be fabricated;

    (c) juxtaposing said elastiomeric microrelief of step (b)
        to a surface;

    (d) causing said material from which an article is to be
        fabricated to polymerize or harden or set;

    (e) removing said elastiomeric microrelief;

    (f) forming or molding a sacrificial material over the
        surface produced in step (d) and causing said
        sacrificial material to polymerize or harden or set
        if necessary;

    (g) repeating steps (b) through (f) until the article
        under fabrication is complete; and,

    (h) removing said sacrificial material added in any step
        (f).